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# Nitrate Contaminant Tracing in Surface and Groundwater in the Great Miami River Watershed: Environmental Isotope Approach

Rachel Kristine Buzeta

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Honors Thesis

Rachel Kristine Buzeta

University of Dayton Geology Department

Zelalem Bedaso, Ph.D.

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## Abstract

The global population has increased exponentially causing several challenges surrounding sustainability, including greater food production needs. To meet these demands and boost agricultural productivity, more efficient practices and fertilizers are used. Synthetic fertilizers and other nutrient sources have resulted in water quality degradation and pollution. Much of the Great Miami River Watershed's streams and aquifers in southwestern Ohio are affected by nitrate contaminants originating from anthropogenic sources including synthetic and organic fertilizer used for agriculture, human wastes (domestic, industrial, and municipal wastes), and urbanization. High nitrate concentrations cause ecological disturbances across all trophic levels. Nitrate levels greater than 10 mg/L also pose a danger to human health, if the contaminant reaches drinking water sources. Water quality monitoring stations report nitrate concentrations in surface and groundwater, but a nitrate contaminant source has not been identified.

Here we used isotope ratios of nitrogen ( $\delta^{15}\text{N}$ ) and oxygen ( $\delta^{18}\text{O}$ ) and boron ( $\delta^{11}\text{B}$ ) in nitrates to identify sources for surface and groundwater. We sampled soil and water from different sites within the Great Miami River catchment. Agricultural sampling sites for water and soil included a city of Dayton owned inorganic farm field, a farm field in Shelby County, and manure from local cows, chickens, and pigs. Soil and water samples from natural sites included forest and grassland landscapes in both Englewood and Germantown Metropark, a forest plot adjacent to Shelby County farm fields, and Estel Wenrick Wetland. Outfall samples were collected from official City of Dayton outfalls in Downtown Dayton and the Lily Creek outfall zone. Wastewater treatment effluent water samples were collected from the Englewood and Miamisburg wastewater treatment plants. For the Summer 2017 field work season, a total of 3 manure, 32 soil samples, and 35 water samples were collected. These samples were analyzed by the University of Utah using an  $\text{AgNO}_3^-$  precipitation method. Boron samples were analyzed at

Stony Brook University using negative ion thermal ionization mass spectrometry. Fall 2018 surface water sampling was primarily done along the Great Miami, Mad, and Stillwater River. Addition samples were taken at City of Dayton outfalls and Miami Conservancy District groundwater monitoring wells. A total of 40 water samples were collected during this field season and they were analyzed at the University of California- Davis Stable Isotope Facility using a bacterial identification assay method.

Using the stable isotope of nitrogen and oxygen we established the unique isotopic composition of different contaminant sources such as agriculture, septic systems, and animal waste. The additional analysis of boron helped to distinguish anthropogenic sources of nitrate from natural sources so that there is greater comprehension of how human activity is affecting water resources within a catchment. Our results show a distinct low  $\delta^{15}\text{N}$  for commercial synthetic fertilizers ( $0.4 \pm 4\text{‰}$ ) and high  $\delta^{15}\text{N}$  for animal and human waste ( $13.0 \pm 1.3\text{‰}$ ). In general, the  $\delta^{15}\text{N}$  from river samples collected during the low river flow lies within a range of human and animal waste, whereas  $\delta^{15}\text{N}$  values of groundwater suggest that the nitrates might have been derived from soil organic matter or synthetic fertilizers. Additional GIS analysis was used to assess nitrate concentration levels within the watershed and a major contamination area, where nitrate levels exceeded 5 mg/L, was identified at the confluence of the 3 rivers and it spans the region downstream, heading southeast. This research provides a regional baseline for nitrate contaminant source tracing and helps to better inform state and local water quality and nutrient management planning based on these findings.

## Acknowledgements

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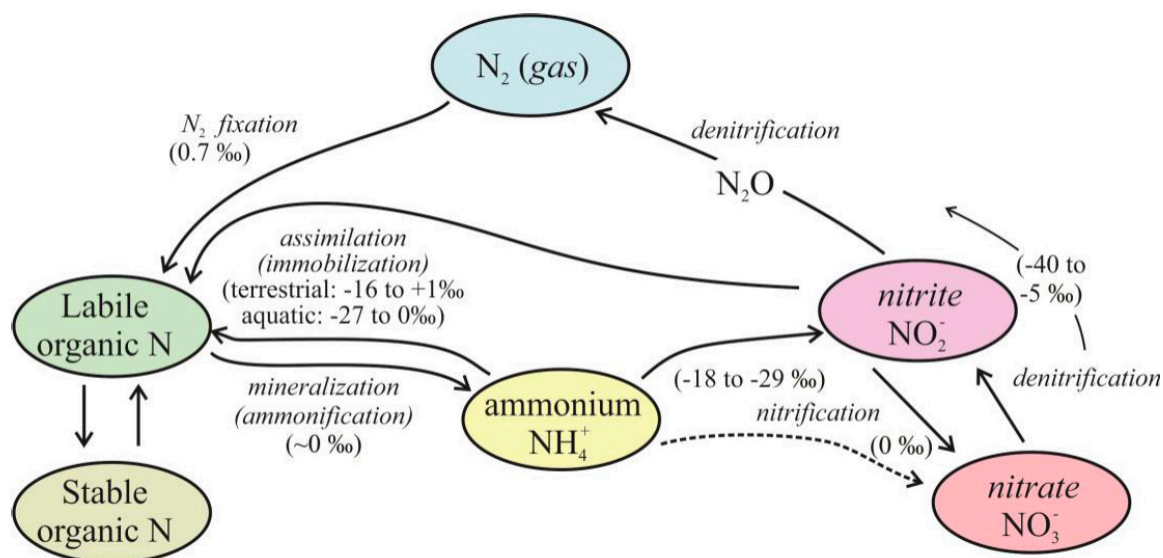
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## 1. Introduction

### 1.1. Nitrate Occurrence

Nitrogen is very abundant gas in the atmosphere as elemental nitrogen composes 78% of air, and it is a vital component of plant life as it makes up 2-4% of plant dry matter and it is an essential macronutrient in plants. As a macronutrient, nitrogen plays an important role in the growth of plants so it serves an important purpose in the biosphere. Microorganisms such as bacteria are responsible for all major conversions of nitrogen between organic and inorganic forms in the biological nitrogen cycle.

The nitrogen cycle allows N to be converted into usable forms. Atmospheric nitrogen is converted into ammonium through bacterial activity, primarily *Rhizobium*, in plant roots. This is a process known as nitrogen fixation. Through mineralization, organic nitrogen becomes an inorganic form like ammonium ( $\text{NH}_4^+$ ). While nitrification is a multi-step oxidation process mediated by several different autotrophic organisms such as chemotrophic bacteria which convert ammonium to nitrite ( $\text{NO}_2^-$ ) to nitrate ( $\text{NO}_3^-$ ) (Sharp, 2017). In assimilation, ammonium and nitrate, mineral forms of nitrogen are converted into organic matter where plants and animals typically preferentially uptake organic nitrogen containing the lighter isotope  $^{14}\text{N}$ . Both nitrite and nitrate are nitrogenous compounds that can be utilized by organisms and excess nitrate can return to atmospheric nitrogen through denitrification processes in poorly aerated soil and anoxic areas (Densmore et al., 2000).



**Figure 1.** Simplified diagram of the nitrogen cycle. The numbers in parentheses indicate average isotopic fractionations ( $\delta^{15}N_{\text{product}} - \delta^{15}N_{\text{source}}$ ) involved with each stage (Sharp, 2017).

As a result of the nitrogen cycle, nitrate is common in soils and organisms. Although nitrates occur naturally in the soil, humans are responsible for contributing large amounts into the soil and water systems. To meet growing food demands and boost agricultural productivity, more efficient practices and fertilizers are used. Use of synthetic and organic fertilizers have resulted in water quality degradation and pollution. Additionally, human wastes resulting from domestic, industrial, and municipal sources, as well as urbanization, contribute significant quantities of nitrates into soil and water that would not otherwise be present.

### 1.2. Nitrogen Isotopes

Nitrogen has two stable isotopes,  $^{14}N$ , the most common form of nitrogen found with a natural abundance of 99.63% and  $^{15}N$  which has an abundance of 0.37%. N-containing compounds on earth show a  $^{14}N/^{15}N$  ratio  $\sim 272$  (Bedard-Haughn et al., 2003). Natural  $^{15}N$  abundance is expressed as  $\delta^{15}N$  in per mill (‰) excess over a standard:



$$\delta^{15}\text{N}(\text{‰}) = \frac{\text{atom}\%^{15}\text{N}_{\text{sample}} - \text{atom}\%^{15}\text{N}_{\text{standard}}}{\text{atom}\%^{15}\text{N}_{\text{standard}}} 1000$$

Calculations of a sample's  $^{15}\text{N}$  value is expressed using the  $R$ -values of the isotope ratios:

$$\delta^{15}\text{N}(\text{‰}) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} 1000$$

Where  $\delta^{15}\text{N}$  (‰) is the sample's isotope ratio relative to the atmospheric air standard.

$R_{\text{sample}}$  and  $R_{\text{standard}}$  are molar ratios of  $^{15}\text{N}$ - $^{14}\text{N}$  (Bedard-Haughn et al., 2003).

Nitrogen also occurs in different oxidation states and various phases. The organic form of nitrogen is important because nearly all isotopic fractionation occurs through “metabolic or metabolically-related processes” (Sharp, 2017). During these biotic processes of nitrogen cycling as a nutrient compound, the lighter nitrogen isotope is preferentially absorbed resulting in a concentration of enriched or more positive  $\delta^{15}\text{N}$  values. Isotope fractionation varies during different processes within the nitrogen cycle as they are kinetically controlled and not equilibrium reactions. The magnitude of isotopic fractionation is variable within each step and dependent on environmental conditions (Sharp, 2017). Nitrate products within the nitrogen cycle will experience a range of -12 to -29‰ change from the original ammonium where it formed (Kendall, 1998).

### 1.3. Nitrate Forensics

The  $\delta^{15}\text{N}$  or the  $^{15}\text{N}$ - natural abundance method is one way stable isotopes can be used to trace the flow of pollution. This method uses the different  $^{15}\text{N}/^{14}\text{N}$  ratios of different pollution sources or their isotopic fingerprint to follow the N source through different media (Bedard-

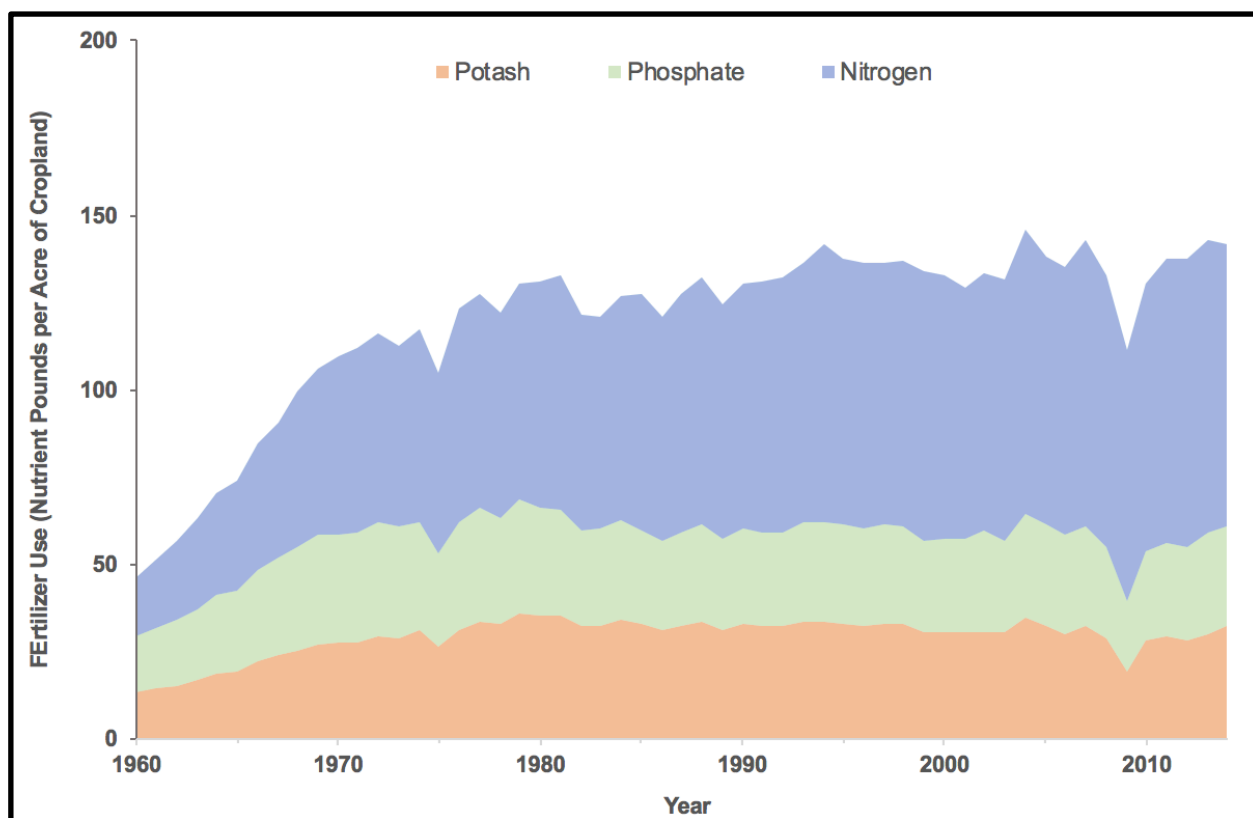
Haughn et al., 2003). Analysis of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  ( $^{15}\text{N}\text{-NO}_3^-$ ,  $^{18}\text{O}\text{-NO}_3^-$ ) was first used for the Sagamon River in 1972 by Kohl et al. In the 1990s,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  analysis grew in popularity as it became recognized as a useful way to identify sources, relative contributions, and pathways of nitrate contaminants in water (Ohte, 2013). This technique has been successful since different sources have exhibited different isotopic compositions or unique “fingerprints” that allow for quantification of the amount of nitrate coming from each potential nitrogen source (Xu et al., 2015).

For example, anthropogenic synthetic fertilizers typically have a  $\delta^{15}\text{N}$  ranging from -4 to +4 ‰, while organic fertilizers have more positive  $\delta^{15}\text{N}$  values from +10 to +20 ‰, and animal wastes typically show  $\delta^{15}\text{N} > 5$  ‰. While some areas may have more defined sources of nitrates or a single known source such as sewer systems, urban areas often struggle to determine natural and human sources contributing to their water systems. Normal chemical analysis of water can only quantify contaminant concentrations and the question regarding contaminant source remains.

#### *1.4. Nitrate Source*

##### *1.4.1. Agriculture*

Growing populations have led to increased food demand and the use of commercial fertilizers to increase crop yields on corporate farms (Fig. 2). The most common types of fertilizer used in the United States for commercial use are nitrogen, phosphate, and potash. These are used for food, feed, fiber and fuel (USDA Economic Research Service, 2018).



**Figure 2.** Commercial fertilizer use in the United States from 1960 to 2014 (Source: United States Department of Agriculture Economic Research Service, USDA ERS, 2018).

Application of organic and inorganic nitrogen showed a dramatic increase during the last fifty years (Mosier et al., 1998). Starting in the 1960s, more acreage of land was used solely for high-yield crop varieties and altered hybrid species that were especially responsive to commercial fertilizer. With modern industrialized farming and livestock raising techniques, this source of nitrates contributes to agricultural runoff problems as excess nutrients flow into rivers and other bodies of water because of nitrate's extremely soluble and mobile nature. The introduction of excess of nitrogen can disturb normal nutrient cycles and disrupt aquatic environments and food chains (Ohte, 2013).

Animal manure, especially cow manure, is another important contributor of nutrients such as nitrogen and phosphorus to water bodies. Nitrogen is capable of existing in various

oxidation states and compounds, and nearly all forms of nitrogen can occur at one point or another within manure. Nitrate and ammonium are two highly soluble compound varieties of nitrogen and as a result, they have great mobility as components of runoff and soil solutions (Follett, 1995). Untreated manure from livestock such as cows, pigs, and chicken are commonly applied to crop-bearing land as a source of nutrients. This raw manure contains nitrogen in the form of organic compounds and ammonium and nitrate. When it rains, these nitrogenous compounds are transported as part of surface runoff which eventually can enter nearby water bodies or leach into the soil as groundwater.

#### *1.4.2. Wastewater*

Most residential homes and businesses in suburban and urban areas have their wastewater managed by local wastewater treatment facilities. The water discharged by these facilities or wastewater effluent typically contains excess nitrogen and phosphorus due to the processing of food, human waste, industrial waste, and various manmade detergents. Wastewater commonly contains nitrogen in the forms of ammonia or urea, but nitrates and nitrites are also included. The presence of highly concentrated nitrogenous compounds found within wastewater effluents have been known to cause a degradation of water quality in receiving bodies of water (Akpore, 2014). Wastewater effluents is one of the primary contributors to water pollution cases, which are not limited to nutrient enrichment. However, the corresponding nutrients from these effluents can stimulate algal growths that may negatively affect water quality. Eutrophication can result in dissolved oxygen depletion, bioaccumulation and biomagnification of contaminants, and increased costs for water purification (Akpore and Muchie, 2011).

#### *1.4.3. Nitrate Source: Atmospheric Deposition*

Nitrogen can also be found in the forms of nitrogen oxides, nitric acid, gaseous ammonia, organic nitrogen, and particulate nitrate and ammonium compounds that circulate through the atmosphere. These atmospheric nitrogenous compounds cycle to both land and water through atmospheric deposition. There are many atmospheric nitrogen pathways such as----, and humans contribute a large amount of nitrogen to the atmosphere, rivalling even natural source contributions in many cases.

Emissions of nitrogen dioxide ( $\text{NO}_2$  or  $\text{NO}_x$ ) have increased exponentially post-industrialization and this has resulted in larger amounts of atmospheric nitrate (Prospero et al, 1996). This is a major anthropogenic effect on nitrogen cycling within both the atmosphere and biosphere (Vitousek et al, 1997). Soil quality and plant biodiversity is affected by the wet or dry deposition of atmospheric  $\text{NO}_3^-$ . As with other forms of nitrate contamination, atmospheric  $\text{NO}_3^-$  can affect the productivity of ecosystems and human health if contamination reaches drinking water sources (Goulding et al., 1998).

#### *1.4.4. Nitrate Source: Soil Organic Nitrogen*

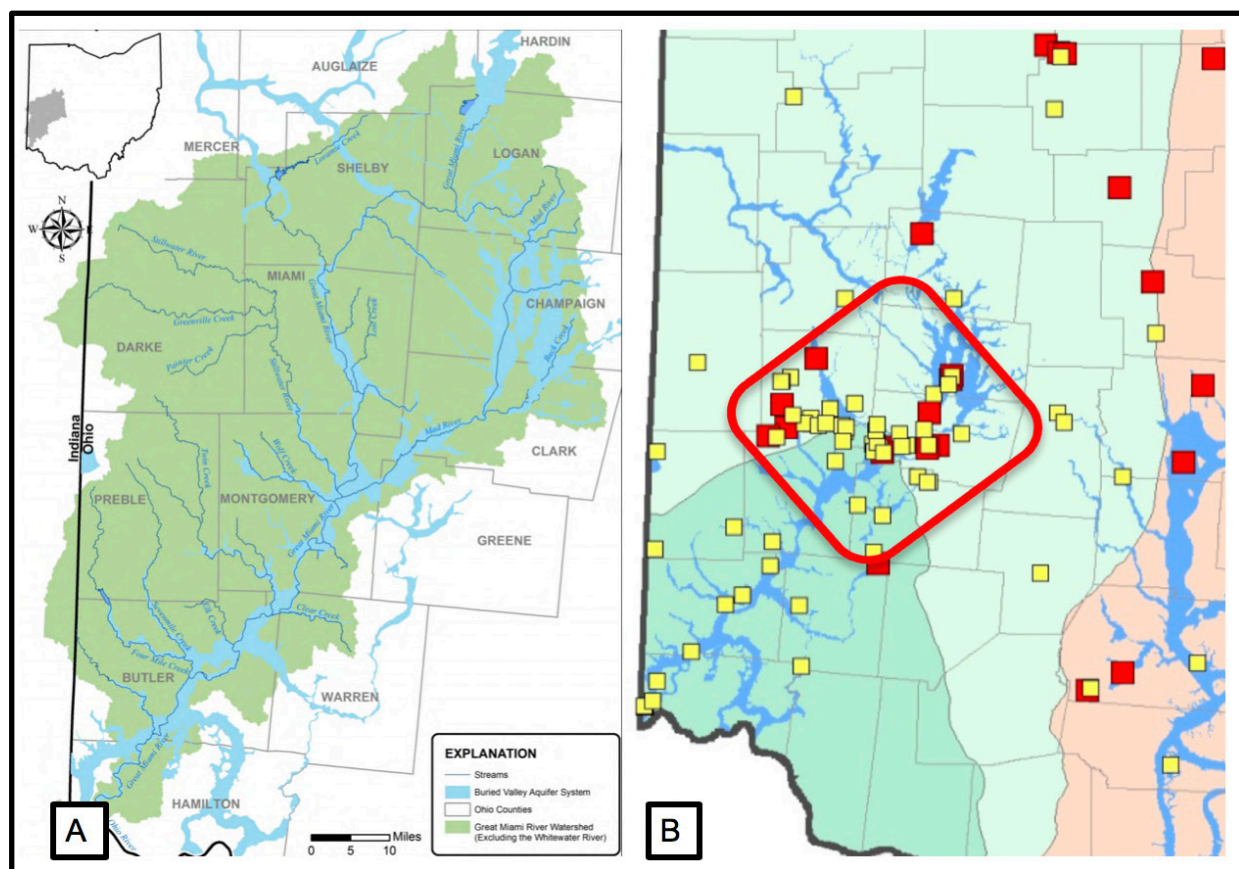
Nitrogen is naturally occurring in soils and is often found in the form of nitrate. Sources of  $\text{NO}_3^-$  in soil may include decomposing plant matter, animal manure, compost, and synthetic fertilizers. Inorganic nitrogen in the form of nitrate is converted into organic forms of nitrogen through microorganisms such as bacteria. After being converted, the organic forms of nitrogen are mobilized and they reenter the soil as a usable component in a plant's diet for growth and production (Follett, 1995). Nitrogen levels within soil is highly variable as they are influenced by factors such as soil type, precipitation amounts, and climate conditions. Soil type is a significant factor which affects nitrate levels as coarsely textured, sand-dominated soils allow for increased

mobility of nitrate in soils. Since water can move more readily in porous soils with coarse textures, soils lose nitrate through leaching increasing likelihood of enriched runoff (Gaines and Gaines, 2008).

### *1.5. Study Area*

Dayton, Ohio is one of the major municipalities located within the Great Miami River Watershed (GMRW) (Fig. 3A). This watershed drains approximately 3,802 square miles. More than 70% of the land in the GMRW is used for agricultural purposes and upwards of 75% of this agricultural land is used for crop production (Debrewer et al., 2000). Dayton is considered part of the lower GMRW by the Ohio EPA (Fig. 3A). This portion of the watershed is 40% urban and residential areas, 28% agricultural land, and approximately 19% forested areas (O EPA, 2017). Application of both organic and inorganic fertilizers rich in nitrate, phosphate, and potash are used throughout the watershed to accelerate the growth of the dominant crops of corn, soybeans, and wheat (Debrewer et al., 2000).

The rivers, streams, and aquifers within the GMRW serve many purposes for the local population. It is a source of recreation, thermoelectric power generation, and a water source for personal, commercial, and agricultural use. While high-quality populations of fish and macroinvertebrate can be found throughout the GMRW, approximately thirty percent of the rivers and streams within the watershed fail to meet the standards for their assigned designation as a “warm-water habitat”, which refers to the assemblage of aquatic organisms and plants typically found provided the land use, local climate, and hydrology (OEPA, 2017).



**Figure 3 A.** Map of the Great Miami River Watershed. The red rectangle is the proposed study area (Map source: MCD). **B.** Distribution of nitrate concentrations in public water system (yellow squares show nitrate >5 but <10 mg/L, red squares show nitrate > 10 mg/L) (Source: EPA 2012 water quality report).

### 1.5.1. National Pollutant Discharge Elimination System (NPDES)

The Clean Water Act which prohibits anybody from “discharging pollutants from a point source into a water of the United States without an NPDES permit. This permit details limits on what types of material can be discharged into a waterway. Monitoring and reporting of discharge is required to ensure safety and quality of water resources. Requirement for such permits enforces state mandatory standards for water and ensures federally required minimums are met in terms of acceptable pollutant levels or parameters. NPDES has a major function in setting wastewater effluent standards (US EPA, 2018).

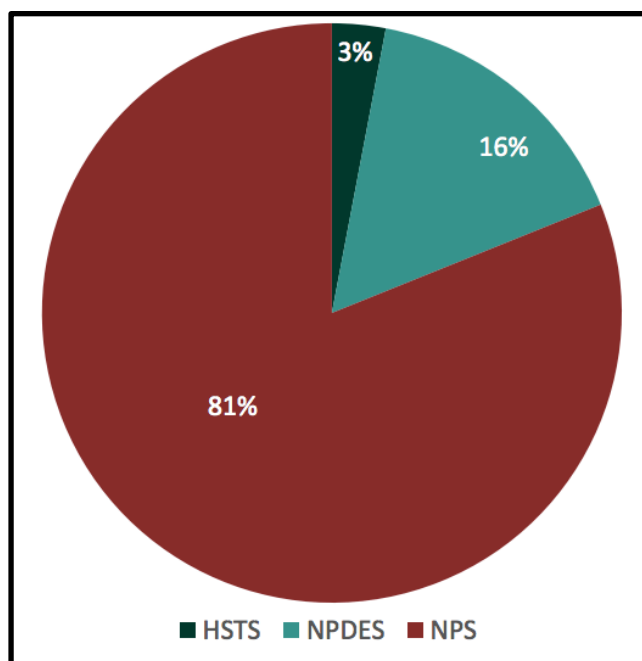
### 1.5.2. EPA Nutrient Mass Balance

The most recent Ohio EPA Nutrient Mass Balance Study of the Great Miami River did not record any statistical trends in the nutrient loading and discharge data, but data comparing contributions of non-point and point sources were collected (Table 1). Overall, non-point sources of pollution have contributed the largest proportion of total P and N at 62% and 81% respectively in 2018 (OEPA, 2018). The National Pollutant Discharge Elimination System (NPDES) loads made up 32% of the total P load and 16% of the total N load. Meanwhile, the Household Sewage Treatment Systems (HSTS) loads totaled to 6% and 3% (OEPA, 2018). Non-point sources such as agricultural runoff and NPDES permitted wastewater effluent seem to be dominant contributors based on these findings (Fig. 4).

Parameter	wy13	wy14	wy15	wy16	wy17
Water Yield (in/yr)	13.6	18.2	15.7	13.2	15.2
20-yr Median Water Yield (in) – 16.1					
<b>Total P</b>					
FWMC (mg/L)	0.36	0.39	0.45	0.27	0.38
Annual Load (mta)	1,230	1,784	1,745	883	1,413
<b>Total N</b>					
FWMC (mg/L)	5.29	4.40	5.31	4.30	5.71
Annual Load (mta)	18,345	20,743	21,486	14,733	22,139

**Table 1.** Annual flow-weighted mean concentration (FWMC), total load and water yield for wy13 through wy17, monitoring sites for the GMRW. Water yield is annual discharge normalized by watershed area (in/yr). FWMC and annual discharge are calculated at the pour point and do not include downstream drainage area (OEPA, 2018).

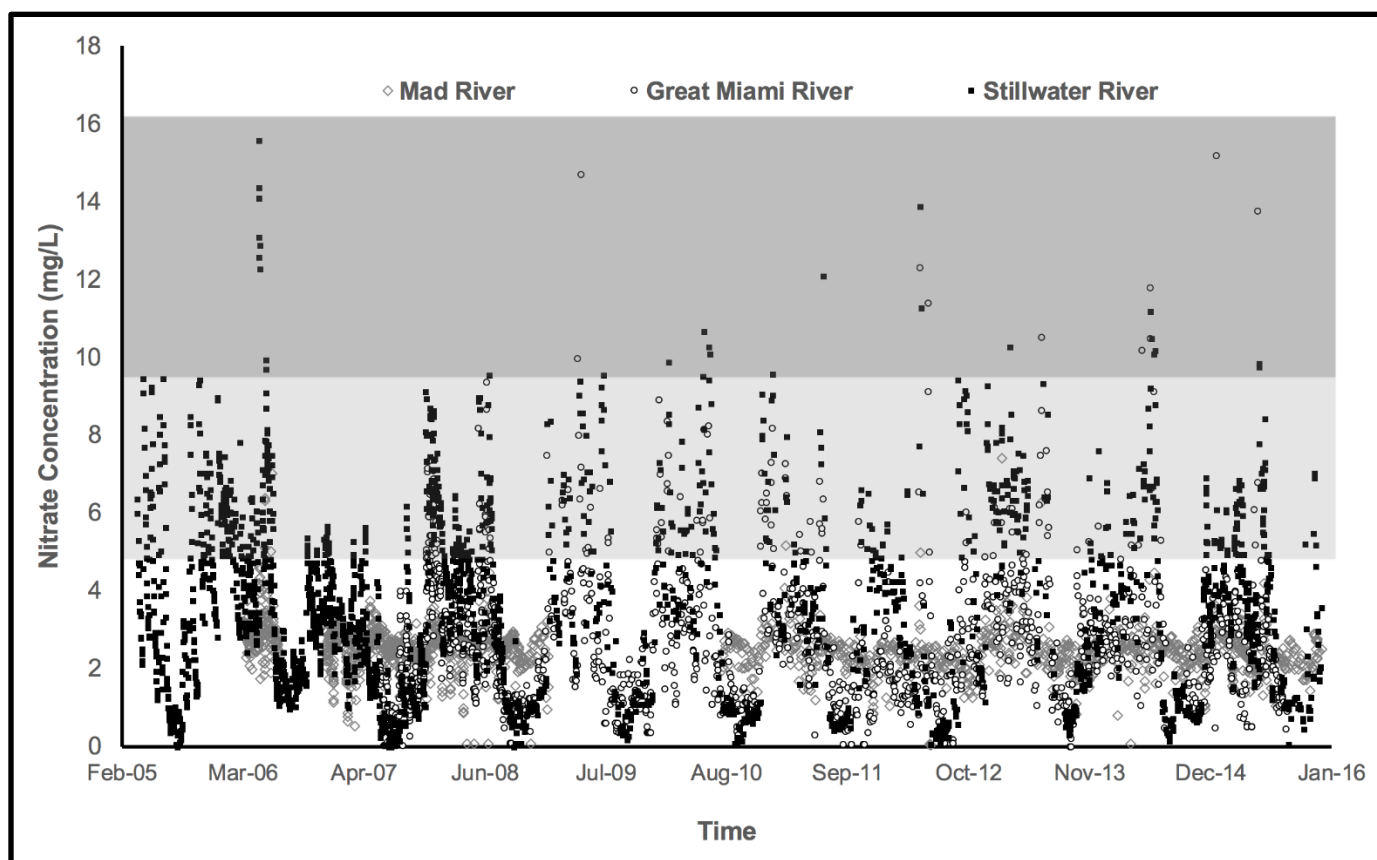




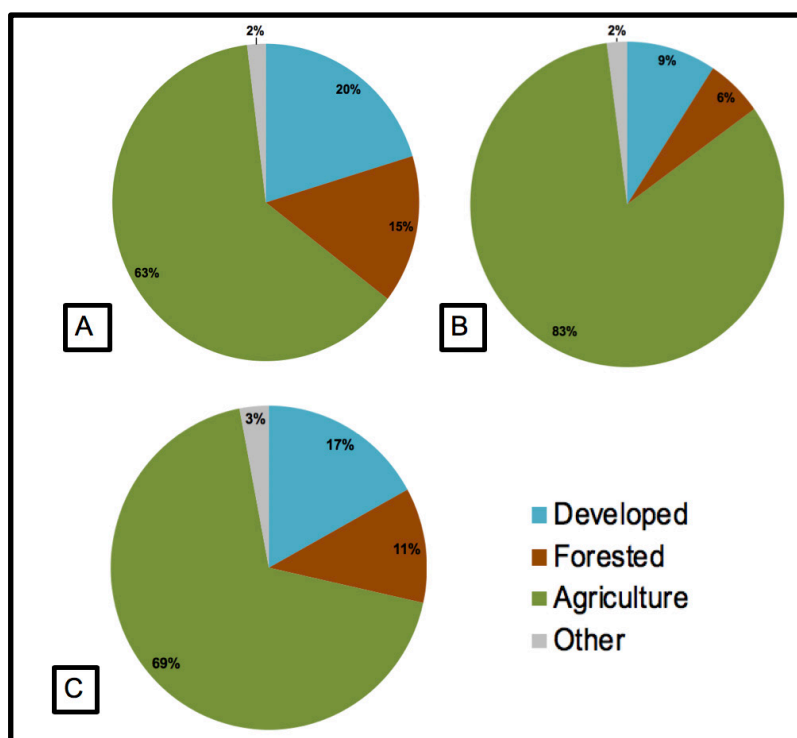
**Figure 4.** Proportion of total nitrogen load from different sources for the Great Miami River watershed, average of 5 years. Sampling done at wy13 through wy17 (OEPA, 2018).

### *1.6. Nitrate Monitoring in Study Area*

Ohio EPA Water Quality reports have shown elevated nitrate levels in public water systems (Fig. 3B). Additionally, on a more local level, surface water monitoring data from the Miami Conservancy District (MCD) in the region has shown elevated nitrate levels mainly  $>5$  mg/L at sampling sites along the Great Miami, Mad, and Stillwater River (Fig. 4). Additionally, storm water sampling conducted by the City of Dayton Department of Water has also recorded several sites of high nitrate concentrations. While it is known that high levels of nitrate contamination are an issue within the Great Miami Watershed, less is known about actual contaminant sources.



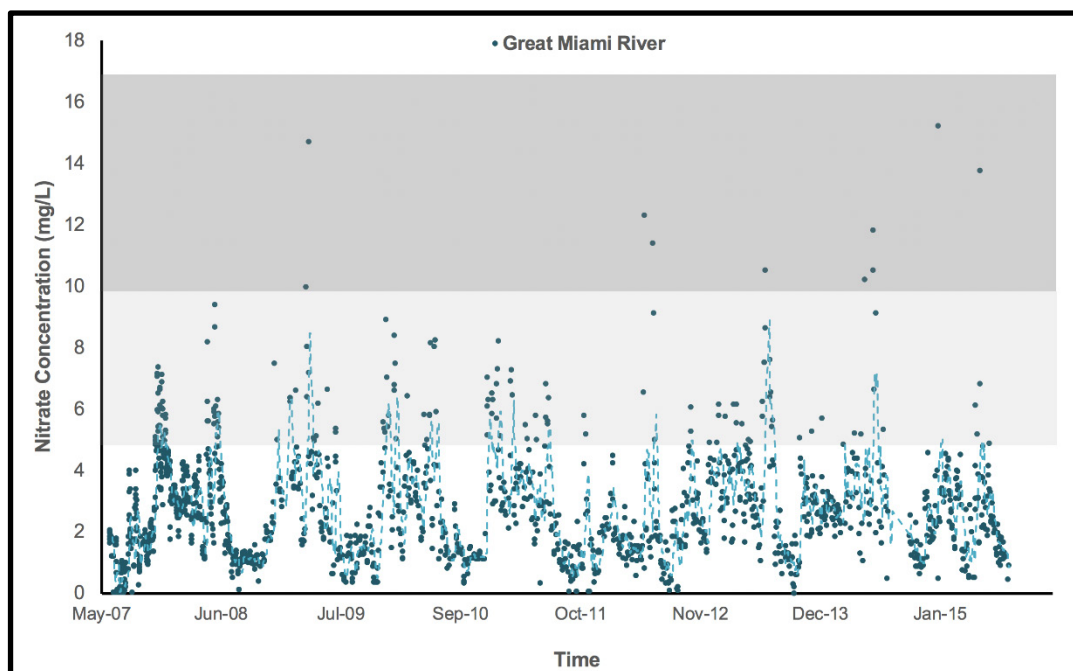
**Figure 5.** Nitrate concentrations at three different sampling stations along the Mad River, Great Miami River, and the Stillwater River over the past 11 years. The light gray box indicates values greater than 5 mg/L. The dark gray indicates values greater than 10 mg/L. Approximately 12% of nitrate concentrations are greater than 5 mg/L (Data source: MCD, 2016).



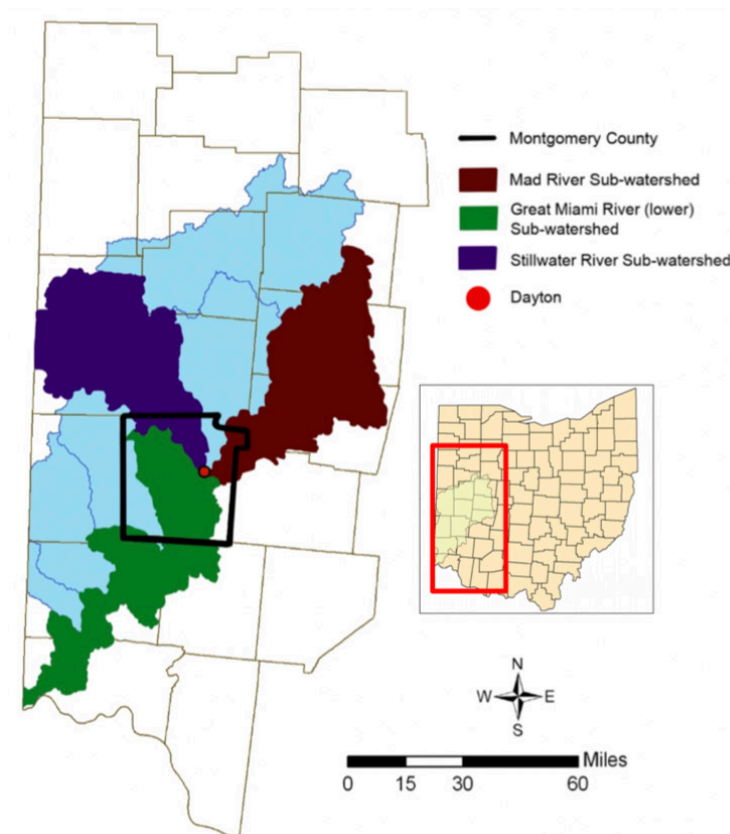
**Figure 6A.** Land usage in the Lower Great Miami River Subwatershed. **B.** Land usage in the Stillwater River Subwatershed. **C.** Land usage in the Mad River Subwatershed (Data Source: MCD, 2016).

### 1.6.1. Lower Great Miami River Subwatershed

According to Ohio EPA reports over the past decade, The Great Miami River and some of its tributaries have displayed good quality, while tributaries in the western portions of the watershed were generally of a lower quality. Sixty-four percent of the monitored sites showed that full aquatic life was supported. Twenty-six percent of the sites only partially supported the expected aquatic life, and the remaining ten percent of sites could not support life. Along this river, aquatic life is typically impaired by alterations to habitats, sedimentation, and nutrient loads. This area has experienced channelization and conversion of local land usage shifts to agriculture and urban or residential developments (OEPA, 2017). Current reports indicate that this subwatershed's land is primarily dedicated to agriculture and urban and residential development (Fig. 6A). Monitoring stations along the Great Miami River have indicated approximately 9.2% of nitrate concentrations measured at sites along the Great Miami River are greater than 5 mg/L, and approximately 0.6% of nitrate concentrations measured are greater than 10 mg/L (Fig. 7).



**Figure 7.** Nitrate concentrations of the Great Miami River from 2007 to 2015. The light gray box indicates values greater than 5 mg/L. The dark gray indicates values greater than 10 mg/L (Data source: MCD, 2016).

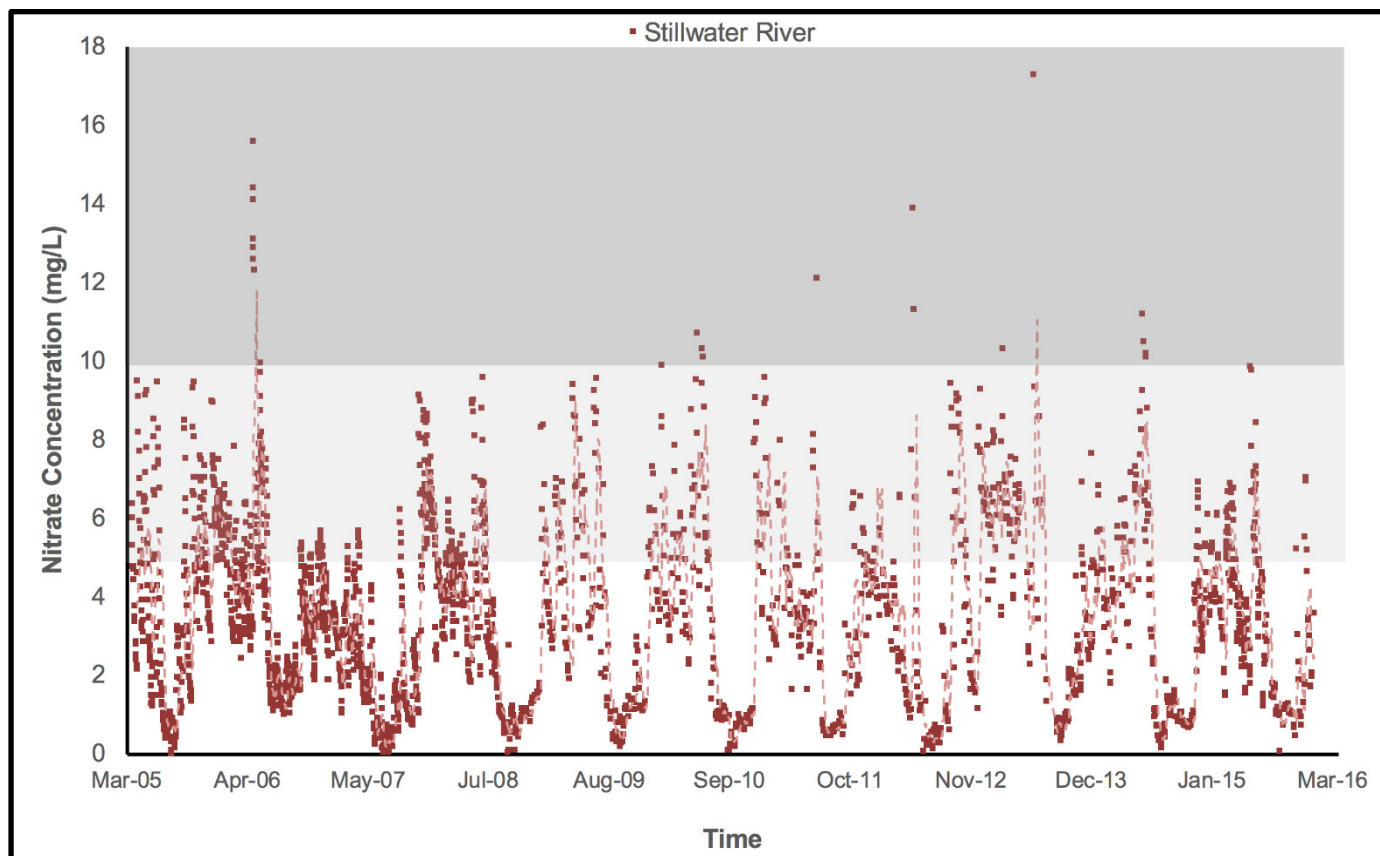


**Figure 8.** Map of Sub-watersheds relevant to this study within the Great Miami River Watershed (Source: OEPA, 2018).

### *1.6.2. Stillwater River Subwatershed*

The Stillwater River spans 67 miles and flows from headwaters in northernmost Darke County to a confluence with the Great Miami River in Dayton. The Stillwater River is part of a sub-watershed that makes up the GMRW (Fig. 3A). Much of the land along the Stillwater River has been physically modified to maintain drainage for row crop agriculture (OEPA, 2017). Historically, almost one-third of this sub-watershed may have been wetlands that has since been reduced to nearly 0.5% its original size due to the implementation of tile drainage and stream channelization. Portions of the land surrounding the Stillwater River, upstream in Darke county,

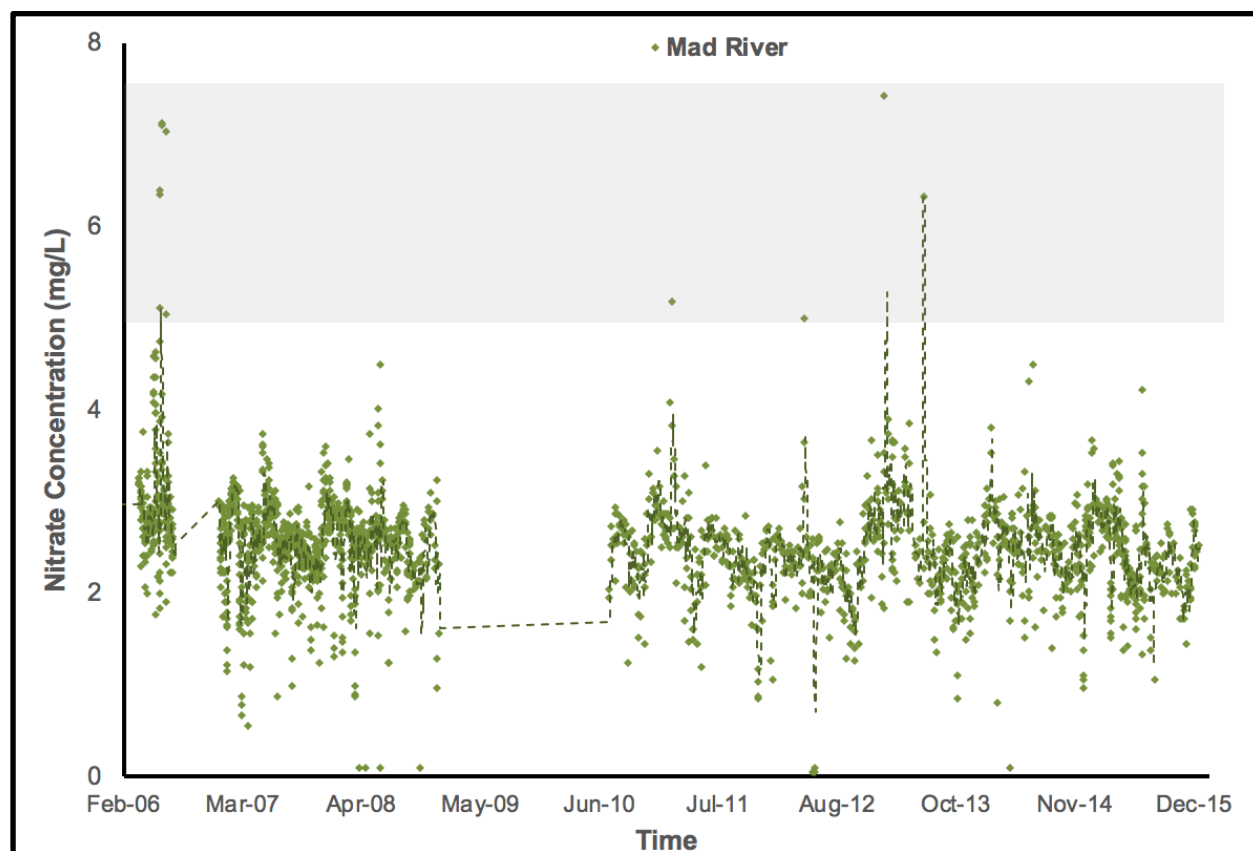
have been identified as having the second highest concentrations of animal feed sites within Ohio. This Subwatershed is dominated by agricultural usage (Fig. 6B).



**Figure 9.** Graph of nitrate concentrations of the Stillwater River from 2005 to 2016. The light gray box indicates values greater than 5 mg/L. The dark gray indicates values greater than 10 mg/L. Approximately 24.4% of nitrate concentrations measured are greater than 5 mg/L, and approximately 0.8% of nitrate concentrations measured are greater than 10 mg/L (Data source: MCD, 2016).

### *1.6.2. Mad River Subwatershed*

Like the Stillwater, the Mad River also converges with the Great Miami River in Dayton. Water quality reports for the Mad River and its sub-watershed have shown elevated nitrate levels associated with agricultural land use and groundwater recharge of surface water sites (Fig. 2). While water quality has not been severely impacted by high nutrient levels, better management practices to minimize nutrient and organic compound release from agricultural and urban areas has been encouraged (OEPA, 2018).

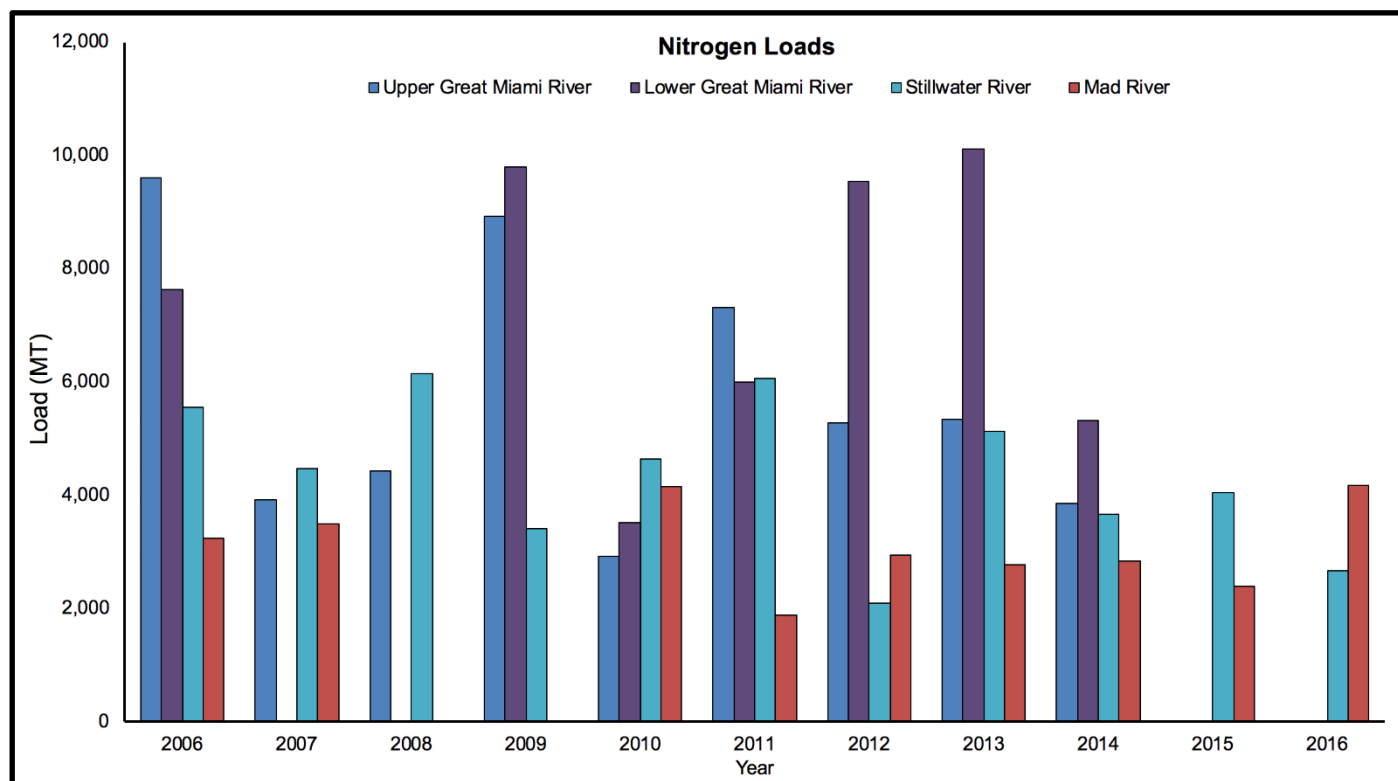


**Figure 10.** Nitrate concentrations of the Mad River from 2006 to 2015. The light gray box indicates values greater than 5 mg/L. Approximately 0.44% of nitrate concentrations measured are greater than 5 mg/L (Data source: MCD, 2016).

### 1.7. Nitrogen Loads

Nitrogen loads are a good indicator of documented nutrient pollution in river bodies. The Great Miami, Stillwater, and Mad River all have substantial nitrogen loads (Fig. 11). The United States Geological Survey's (USGS) Spatially Referenced Regression on Watershed Attributes (SPARROW) model uses a mass-balance approach to estimate excess amounts of nutrients exported from watersheds like the GMRW and how much they deliver downstream. Ohio is ranked #4 nationally in terms of net incremental total nitrogen (TN) load measured in 1000 kg/yr with approximately 231,831 (OEPA, 2018). The Mad River and Stillwater River converge with the Great Miami and it joins the Ohio River past Cincinnati. This is important to keep in mind as

the Ohio River is a tributary of the Mississippi River and a huge anoxic zone is found at the Gulf downstream due to nutrient overloading from upstream waterbodies such as the Great Miami.



**Figure 11.** Nitrogen loads from 2006 to 2016 (Data source: MCD).

### *1.8. Wastewater Treatment Plants*

Wastewater can be defined as water that has constituents of human and/or metabolic wastes or water that has residual material from cooking, cleaning, and/or bathing (Buchanan, 2019). Collection and treatment of sewage and wastewater is a necessary part of public health. Municipal wastewater treatment plants are responsible for the processing of raw wastewater. It undergoes preliminary, primary, and secondary treatment to form effluent and sludge for utilization or disposal. Primary treatment is focused on removal of approximately 60% of suspended solids from wastewater. Secondary treatment provides dissolved oxygen (DO) to aerobic microorganism and works to remove about 85% of organic matter by activating present

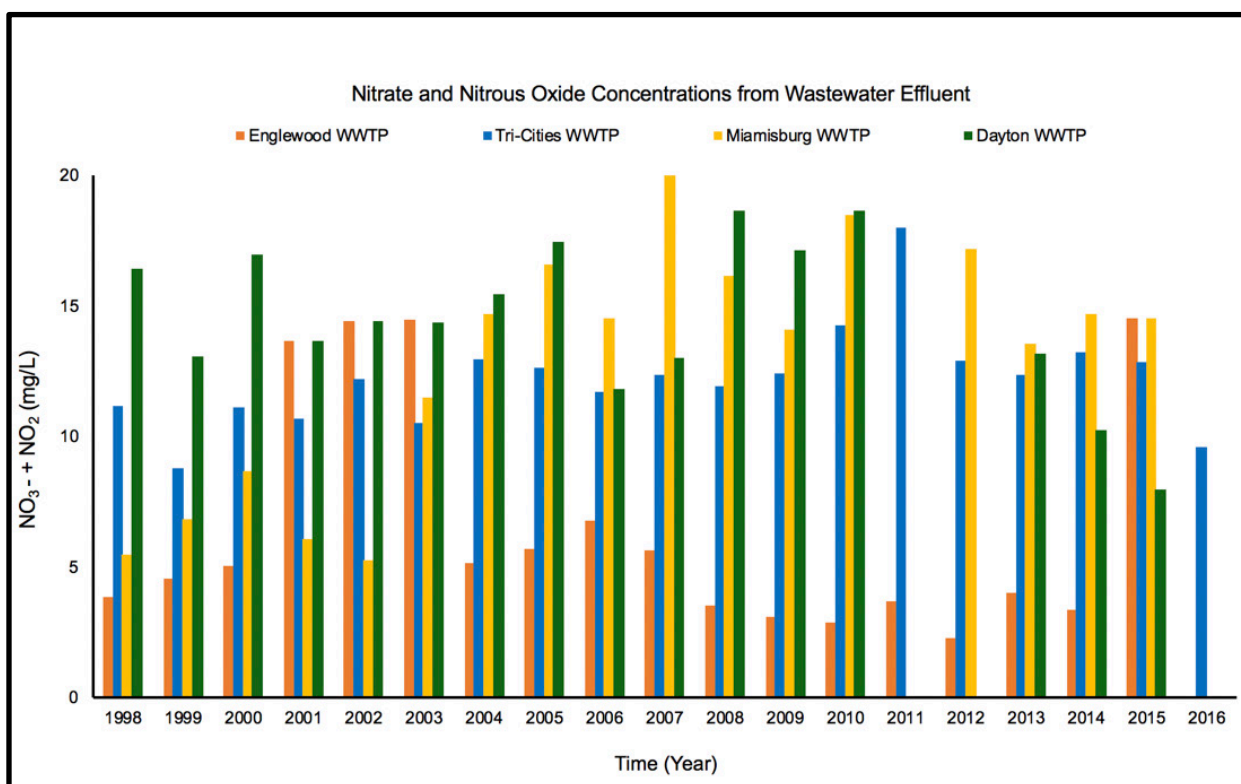
bacteria. Chlorine is used for elimination of pathogenic bacteria and reduction of odor.

Chlorination successfully eliminates 99% of harmful bacteria in effluent (Buchanan, 2019).

NPDES permits establish the discharge limits and conditions for effluent released from plants into surface water such as rivers. Standards that are included and must be followed relate to five-day biochemical oxygen demand (BOD5), total suspended solids (TSS) removal, and pH.

Wastewater effluent, while it has standards regulating it, contains high concentrations of nutrients such as phosphorus and nitrogen (OEPA, 2018).

Local water treatment plants use conventional calcium oxide or lime processing. The pH of the water is adjusted using CO<sub>2</sub> post-softening. The water undergoes fluoridation then chlorination. Sand filtration is the final step in local wastewater treatment (City of Dayton Department of Water, 2018). Data shows that local wastewater treatment plants operating within their NDPES permit are releasing effluent enriched with nitrogen, in the form of nitrate and nitrous oxide, with most levels greater than 5 mg/L (Fig. 12).



**Figure 12.** Graph of nitrate and nitrous oxide concentrations in wastewater effluent over time (Data source: MCD, 2016).



## 2. Methodology

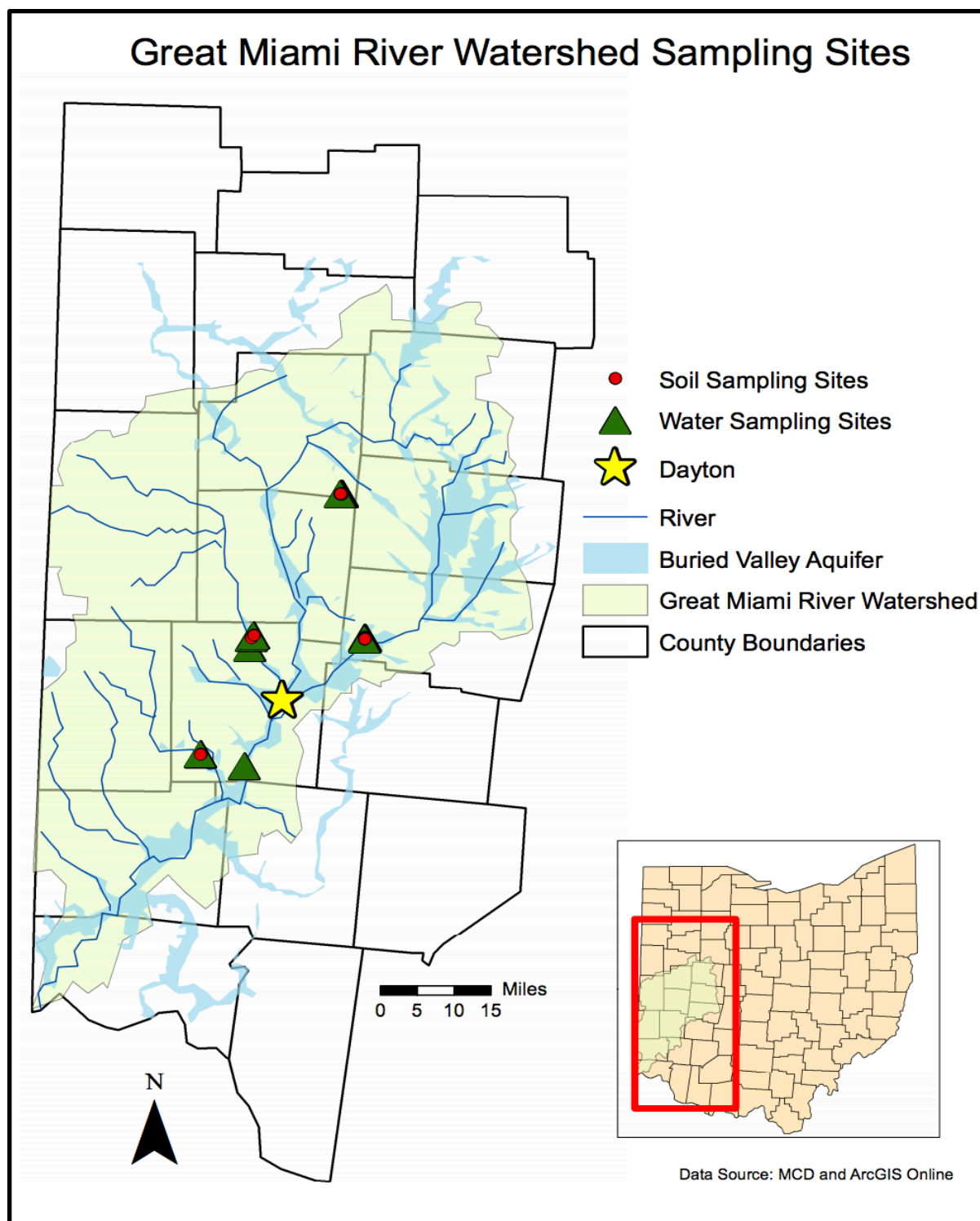
### 2.1. Summer 2017 Sampling

#### 2.1.1. Soil Sample Collection

Soil samples were taken with an auger in 10 cm intervals. Sample depths ranged from 10 to 30 cm. Sample sites were chosen based off of land usage sites including, farm sites with a recent history of organic fertilizer use and synthetic fertilizer use. Additionally, samples from natural sites such as forests, open grassland areas, and wetlands. Soil samples were characterized based off color when wet and dry, texture (clay, silt, sand content), and presence of rootlets and vegetation. Samples were stored in Hubco Cloth Geological Sample Bags and labelled. A total of 32 soil samples were collected. After collection, samples were allowed to dry and additional notes were taken if applicable. Rootlets and vegetation were picked out using tweezers cleaned

**Figure 12.** Graph of nitrate and nitrous oxide concentrations in wastewater effluent over time (Data source: MCD).

Manure from pigs, cows, and chickens were also collected. Rootlets were picked out and samples were dried. They were homogenized using a mortar and pestle that was cleaned with DI water and alcohol between the crushing of each sample.



**Figure 13.** Map of preliminary sampling sites from Summer 2017.

### *Direct Estimation of Organic Matter by Loss on Ignition (LOI)*

Transference of samples was done using tools cleaned with alcohol between samples. Weight percent of organic matter and carbonate content of soils is based on sequential heating in an oven (Dean, 1974).

Porcelain crucibles were weighed and about 1 gram of each sample was taken and weighed within the crucible and total weights were recorded in grams. These samples were placed on a tray and heated at 105°C for approximately 12 hours. This step is to determine water content. After being removed from the oven, samples were placed in a desiccator to cool. Once the crucibles cooled down, samples were weighed again and values were recorded. Next, samples were heated at 550°C for two hours, cooled in desiccator, and weighed. LOI was calculated using the following equation:

$$LOI_{550} = ((DW_{105} - DW_{550}) / DW_{105}) * 100$$

Where  $DW_{105}$  represents dry weigh before combustion and  $DW_{550}$  represent weight after heating, both are measured in grams. The weight difference represents the organic carbon contained in each sample (Dean, 1974). Samples were heated again at 950°C for two hours, cooled in desiccator, and weighed. This caused the removal of carbon dioxide from carbonates in the soil samples. Carbonate content was calculated using the following equation:

$$LOI_{950} = ((DW_{550} - DW_{950}) / DW_{105}) * 100$$

Where  $DW_{950}$  is the dry weight of each sample after heating to 950°C.

### *Laboratory Work*

Soil samples were crushed and homogenized into a fine powder using a mortar and pestle. They were homogenized to insure the subsample is representative of average isotopic

composition. Any remaining rootlets were removed using tweezers cleaned with alcohol between samples. The mortar and pestle used was also rinsed with DI water and cleaned with alcohol between each sample. Homogenized samples were labelled and stored in sterilized plastic tubes.

Carbon and nitrogen analysis for these solid samples were conducted by the SIRFER lab of the University of Utah. Measurements of C and N isotope ratios of organic and inorganic solid materials using an isotope ratio mass spectrometer (IRMS) along with an elemental analyzer (EA) in continuous flow (CF) mode. Specific instrumentation for this lab is Finnigan Mat Delta+ Advantage or Finnigan Mat Delta+ IRMS, Carlo Erba CHN EA1110, and ThermoFinnigan Conflo III (SIRFER, 2018).

In the EA, Samples are combusted at 1080°C in a reactor packed with copper oxide and tungsten (VI) oxide. Following combustion, oxides are removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flows through a water trap (magnesium perchlorate). N<sub>2</sub> and CO<sub>2</sub> are separated using a molecular sieve adsorption trap before entering the IRMS. During analysis, samples are interspersed with several replicates of at least two different regulated standards to ensure accuracy. The long term standard deviation is 0.2 ‰ for <sup>13</sup>C and 0.3 ‰ for <sup>15</sup>N. The final delta values were expressed relative to international standards VPDB (Vienna PeeDee Belemnite) and Air for carbon and nitrogen, respectively (SIRFER, 2018).

### *2.1.2. Water Sample Collection*

Water samples were collected from different land usage sites and sources including farmland, wetland, forest areas, wastewater effluent, storm water outfalls, and runoff. When available, samples were initially tested with dip strips that provided a very rough estimate of the nitrite and nitrate concentration of the water being sampled in mg/L. Water parameters were also

collected using a YSI Sonde at each site. This provided information such as dissolved oxygen content (DO), pH, conductivity, temperature, and oxidation reduction potential (ORP).

When sites and water sources were accessible, samples were taken using HSW Norm-Ject Sterile Luer-Lock Air-tite 50 mL Syringes. Different syringes were used to collect each sample and they were stored in narrow mouth high-density polypropylene 500 mL (16.9 oz) bottles. Samples bottles were labelled and sealed with parafilm. Samples were kept in a cooler with ice to keep them around 4°C. this limits microbial activity that could compromise the samples and nitrate concentrations.

Samples were refrigerated until they were filtered using a 25mm diameter, 0.8/0.2  $\mu\text{m}$  sterile acrodisc filters with supor membrane. This ensures that all bacteria and particulates are removed from samples.

#### *AgNO<sub>3</sub><sup>-</sup> Precipitation Methodology*

For the analysis of water samples, the University of Utah purified nitrate samples using anion exchange resin and precipitated silver nitrate (AgNO<sub>3</sub>). This technique requires aqueous solutions to have nitrate concentrations greater than 50  $\mu\text{mol}$ . Graphite power was added to AgNO<sub>3</sub> precipitates and placed into a flame sealed 6mm quartz tube. Samples were heated at 860 °C for 3 hours for high temperature combustion. Measurements of  $\delta^{18}\text{O}$  were made using a mass spectrometer that analyzed carbon dioxide converted from the nitrate-oxygen. Measurements of  $\delta^{15}\text{N}$  were made based on N<sub>2</sub> and CO<sub>2</sub> production in an elemental analyzer and an isotope ratio mass spectrometer set in continuous flow mode (Mayer et al., 2002). This method has some negatives in terms of application: (1) it requires a greater nitrate concentration (>50  $\mu\text{mol}$ ) to avoid contamination from atmospheric nitrogen, (2) the chemical reagents used in this methodology are expensive, (3) other ions present such as Cl<sup>-</sup> can influence quantification of

nitrate. However, this method is able to operate at a high level of sensitive, no preservatives are added that may compromise the integrity of the sample, preservation time under proper conditions is extensive (up to 1 year), and this is well-known, well-tested method (Zhang et al., 2018)

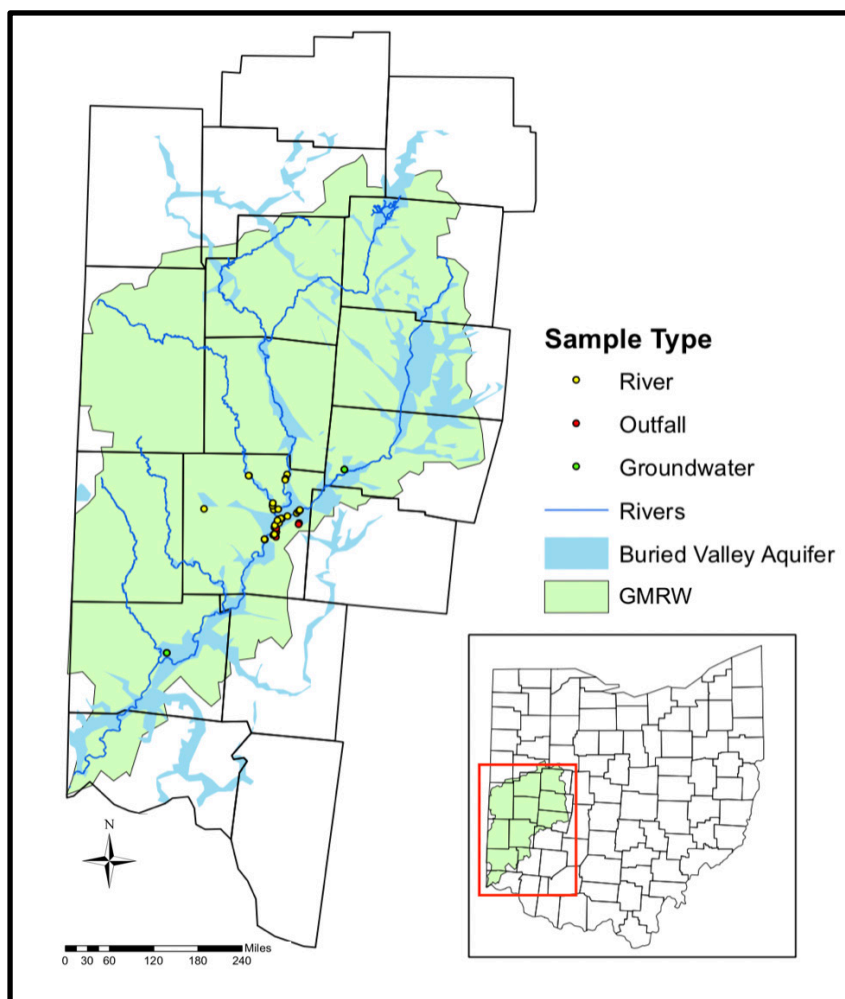
### *Boron Analysis*

Samples were chosen to be representative of a variety of potential contamination sources. Liquid samples included two effluent samples from wastewater treatment plants and two runoff samples. Solid samples sent were subsamples from homogenized soils and homogenized manure. Two farm soils, two forest samples, and three manure samples were sent for analysis at Stony Brook University. A negative ion thermal ionization mass spectrometry (NTIMS) was used. Samples are ionized and the ratio of  $^{11}\text{B}/^{10}\text{B}$  is determined.

## *2.2. Fall 2018 Sampling*

### *2.2.1. River & Stormwater Sites*

The Great Miami, Stillwater, and Mad River were chosen for this study as they are three major rivers within the Greater Dayton Area and they converge in Downtown Dayton. Fourteen samples were taken from the Great Miami River, ten from the Mad River, and five from the Stillwater River. Additionally, four storm water outfalls within the jurisdiction of the City of Dayton Department of Water (Fig. 14).



**Figure 14.** Map of sampling sites from Fall 2018.

### 2.2.2. Nitrate Analysis

For this project, thirty water samples were collected from surface and groundwater from the Great Miami River, Mad River, Stillwater River and storm water drainage sites in the early fall. When sites and water sources were accessible, samples were taken using HSW Norm-Ject Sterile Luer-Lock Air-tite 50 mL Syringes. Different syringes were used to collect each sample and they were stored in narrow mouth high-density polypropylene 500 mL (16.9 oz) bottles. Samples bottles were labelled and sealed with parafilm. Samples were kept in a cooler with ice to keep them around 4°C. this limits microbial activity that could compromise the samples and nitrate concentrations. Samples were refrigerated and they were filtered using Acrodisc sterile glass fiber syringe filters with Supor membrane (25 mm diameter, 0.2  $\mu$ m pore space). This

filtration removed rootlets and bacteria. To prevent samples from being compromised, they were refrigerated and stored at 4°C to prevent microbial activity before being frozen.

Pace Analytical Englewood Labs conducted lab analysis on water sample duplicates which contained sulfuric acid (2 mL H<sub>2</sub>SO<sub>4</sub> per liter) and were preserved by refrigeration at 4°C. They used a spectrophotometric cadmium reaction. In this method, samples are run through a cadmium column causing a reduction reactions where nitrate is reduced to nitrite. An added reagent reacts to the nitrites within a sample to produce a red-colored solution. This resulting solution was measured using a spectrophotometer at a wavelength of 543 nanometers (nm). The absorbance of each sample's solution was measured and the concentration of the nitrite was directly proportional to the absorbance reading.

### *2.2.3. Bacterial Denitrification Methodology*

Nitrate analysis for water samples was conducted by University of California- Davis Stable Isotope Facility using bacterial denitrification assay. Ratios of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  were measured using a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (IRMS). Gas samples are purged from vials through a double-needle sampler into a helium carrier stream at 25 mL/min. The gas sample passed through a CO<sub>2</sub> scrubber (Ascarite) and nitrous oxide (N<sub>2</sub>O) was contained and concentrated in two liquid nitrogen cryo-traps operated in series so the N<sub>2</sub>O is held in the first trap until the non-condensing portion of the sample gas has been replaced by helium carrier, then passed to the second, smaller trap. Finally, the second trap is warmed to ambient, and the N<sub>2</sub>O is carried by helium to the IRMS via an Agilent GS-Q capillary column (30m x 0.32 mm, 40°C, 1.0 mL/min). This column separates N<sub>2</sub>O from residual CO<sub>2</sub>. A reference N<sub>2</sub>O peak is used to calculate provisional isotope ratios of the sample N<sub>2</sub>O peak. Final  $\delta^{15}\text{N}$  values are



calculated by adjusting the provisional values such that correct  $\delta^{15}\text{N}$  values for laboratory reference materials are obtained. The calibration standards are the nitrates USGS 32, USGS 34, and USGS 35, supplied by NIST (National Institute of Standards and Technology, Gaithersburg, MD). Additional laboratory reference materials are included in each batch to monitor and correct for instrumental drift and linearity (UC Davis SIF, 2018).

A downfall of this method is the inability to distinguish between nitrate and nitrite in a water sample. Very accurate nitrate level reports are necessary in assuring the quality of the results. Removal of nitrite via separate methodology is necessary for the success of this method. Additionally, bacterial cultivation requires a long time and the choice of bacteria cultured has an effect on the transformation of nitrate into  $\text{N}_2\text{O}$  (Zhang et al., 2018). Despite these limitations, the denitrifier method is the standard method used by the United States Geological Survey.

### **3. Results**

#### *3.1. Regional Isotopic Signatures*

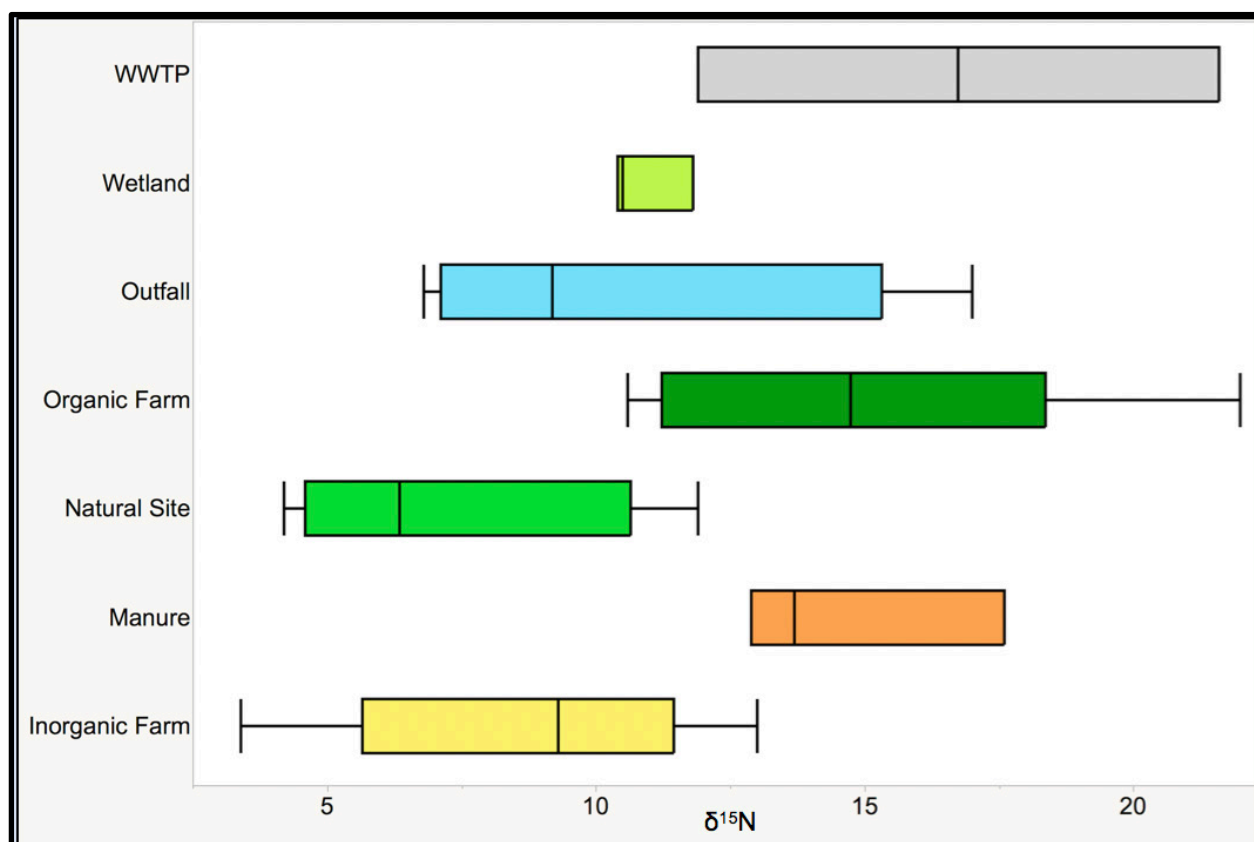
Based on the results of our soil and water sample analysis, our data demonstrates the principle that different nitrate sources (end members) will exhibit different isotopic compositions with limited variability. Isotopic signatures for both  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  were constructed for each land usage type and nitrate source (natural site/forested area, wetland, organic farm, inorganic farm, outfall, wastewater treatment plant (WWTP), and manure) (Fig. 15a and 15b). Values of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  were plotted based on source. Additionally,  $\delta^{15}\text{N}$  values were plotted against  $\delta^{18}\text{O}$  to understand where the data fit into previously published isotopic signature fields for the different nitrate contaminants. The dual tracing method was successful in providing a wider

range of separation of isotopic signatures since  $\delta^{15}\text{N}$  values from different sources can overlap. Overall, the data resulting from the summer 2017 sampling was coherent with previously published values from other case studies (Table 2). Our data falls within a reasonable range of previously collected data and our isotopic signatures and ranges show some regional variability.

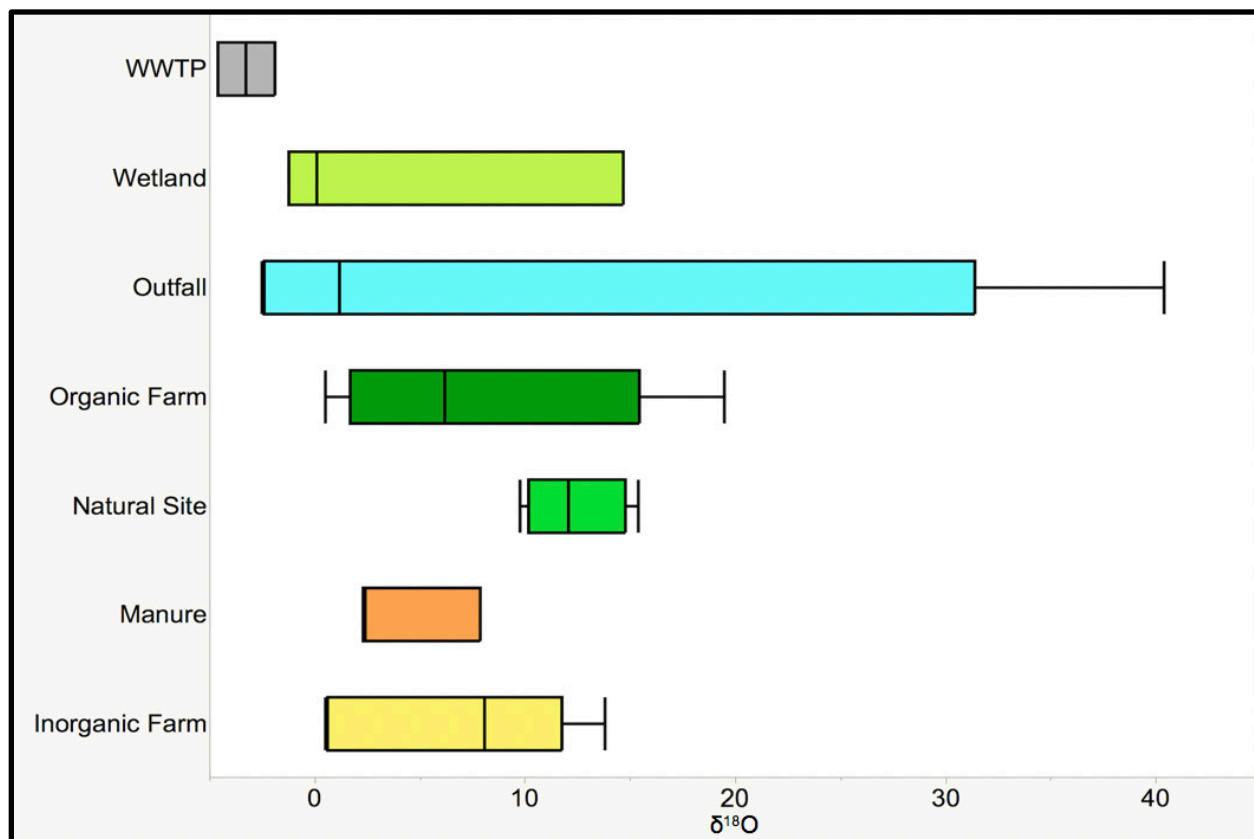
Additional analysis boron isotope ( $\delta^{11}\text{B}$ ) was used to differentiate organic and anthropogenic sources that have an overlap in the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  space. Values of  $\delta^{15}\text{N}$  and  $\delta^{11}\text{B}$  were plotted based on source (WWTP, natural site, or manure). Values of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  were also plotted based on source. Data successfully displays distinct isotopic signatures for natural and anthropogenic contamination sources. Our result shows a distinct low  $\delta^{15}\text{N}$  for commercial synthetic fertilizers ( $0.4 \pm 4\text{‰}$ ) and high  $\delta^{15}\text{N}$  for animal and human waste ( $13.0 \pm 1.3\text{‰}$ ) (Table 2).

Potential Contaminant Source	<u>Previous Data</u>		<u>Our Data</u>	
	$\delta^{15}\text{N}$ (‰)	$\delta^{18}\text{O}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{18}\text{O}$ (‰)
Commercial Fertilizer	-4 to +4	+18 to +26	+3 to +13	> 0
Animal or Human Waste	> +10	-4 to +12	+9 to +14	-4 to +8
Precipitation	-3	+18 to +60	> +5	> +30
Organic Nitrogen/ Soil Nitrogen	+4 to +9	-4 to +1	+4 to +12	-1 to +16

**Table 2.** Summary table comparing previous data for typical  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in dissolved nitrate ( $\text{NO}_3^-$ ) from different nitrate sources to results from the preliminary stage of this study. (Source: Motzer, 2006).

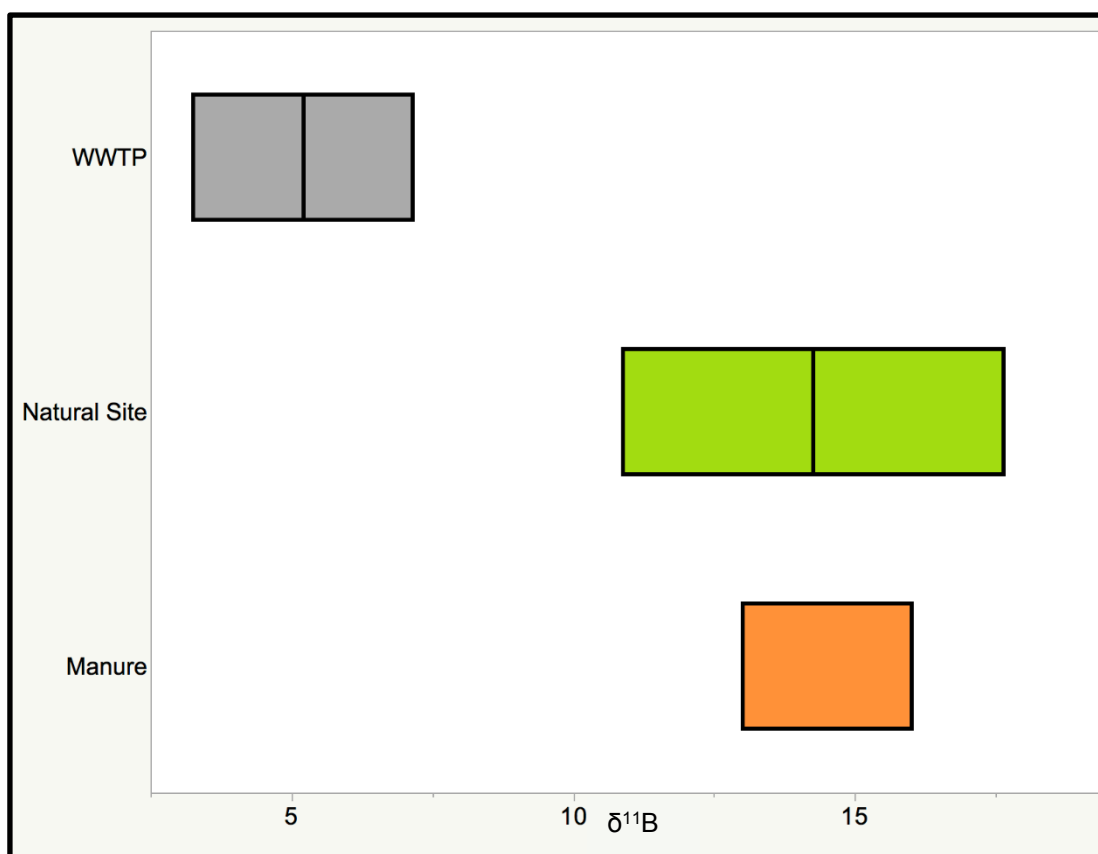


**Figure 15a.** Nitrogen isotopic signatures for different land usage sites. WWTP represents wastewater treatment plants.

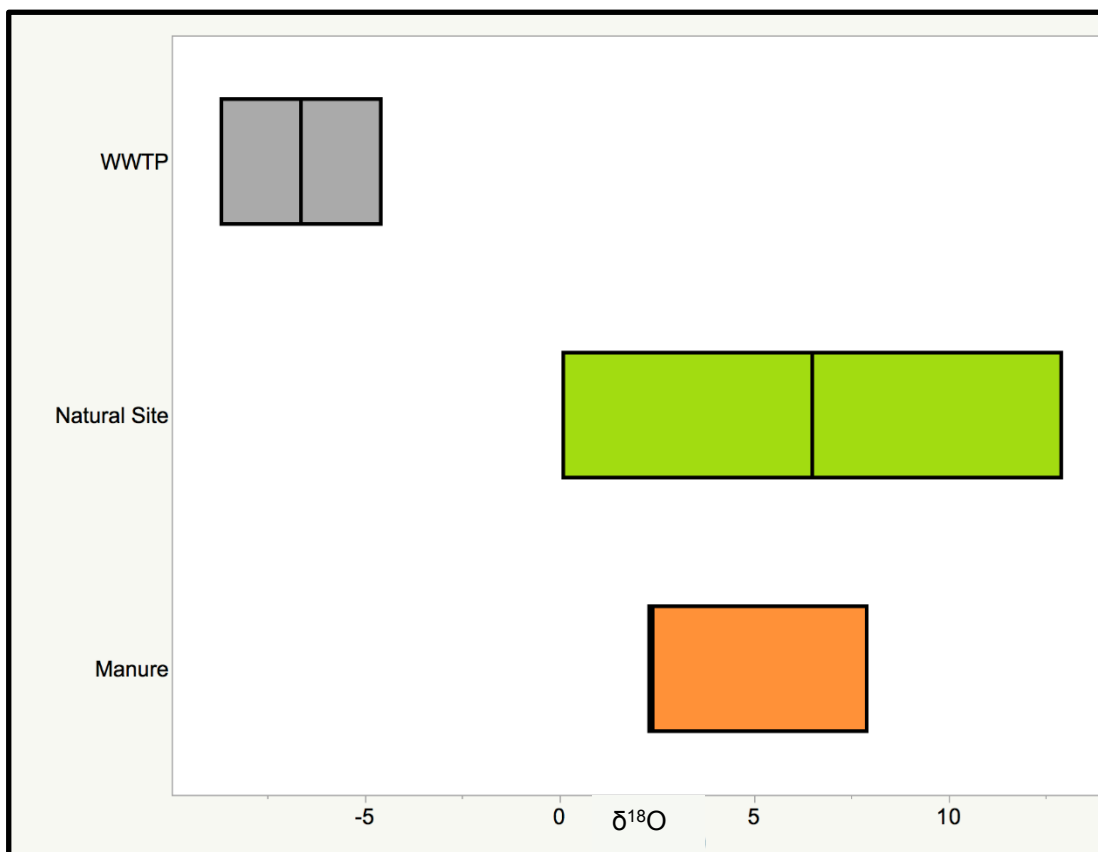


**Figure 15b.** Oxygen isotopic signatures for different land usage sites. WWTP represents wastewater treatment plants.

For WWTP, isotopic ranges were tightly between +9.9‰ and +11.9‰ for  $\delta^{15}\text{N}$  and between -8.7‰ and -4.6‰ for  $\delta^{18}\text{O}$ . Wetland samples isotopically ranged between +10.4‰ and +11.8‰ for  $\delta^{15}\text{N}$  and between -1.2‰ and +14.7‰ for  $\delta^{18}\text{O}$ . Outfalls had a broad range of +6.8‰ and +17‰ for  $\delta^{15}\text{N}$  and between -2.1‰ and +4.5‰ for  $\delta^{18}\text{O}$ . Organic farm samples had more enriched isotopic ranges between +12.9‰ and +22‰ for  $\delta^{15}\text{N}$  and between +2.3‰ and +7.9‰ for  $\delta^{18}\text{O}$ . Natural sites, such as forested areas and prairie-like locations, isotopically ranges between +4.2‰ and +11.9‰ for  $\delta^{15}\text{N}$  and between +9.8‰ and +15.4‰ for  $\delta^{18}\text{O}$ . Manure samples of pig, cow, and chicken, isotopically ranges between +12.9‰ and +17.6‰ for  $\delta^{15}\text{N}$  and between +2.3‰ and +7.9‰ for  $\delta^{18}\text{O}$ . Lastly, inorganic farm samples range ranged between +3.4‰ and +18.8‰ for  $\delta^{15}\text{N}$  and between -0.5‰ and +19.5‰ for  $\delta^{18}\text{O}$ .



**Figure 16a.** Boron isotopic signatures ( $\delta^{11}\text{B}$ ) for boron samples separated by source. WWTP represents wastewater treatment plants.



**Figure 16b.** Oxygen isotopic signatures ( $\delta^{18}\text{O}$ ) for boron samples separated by source. WWTP represents wastewater treatment plants.

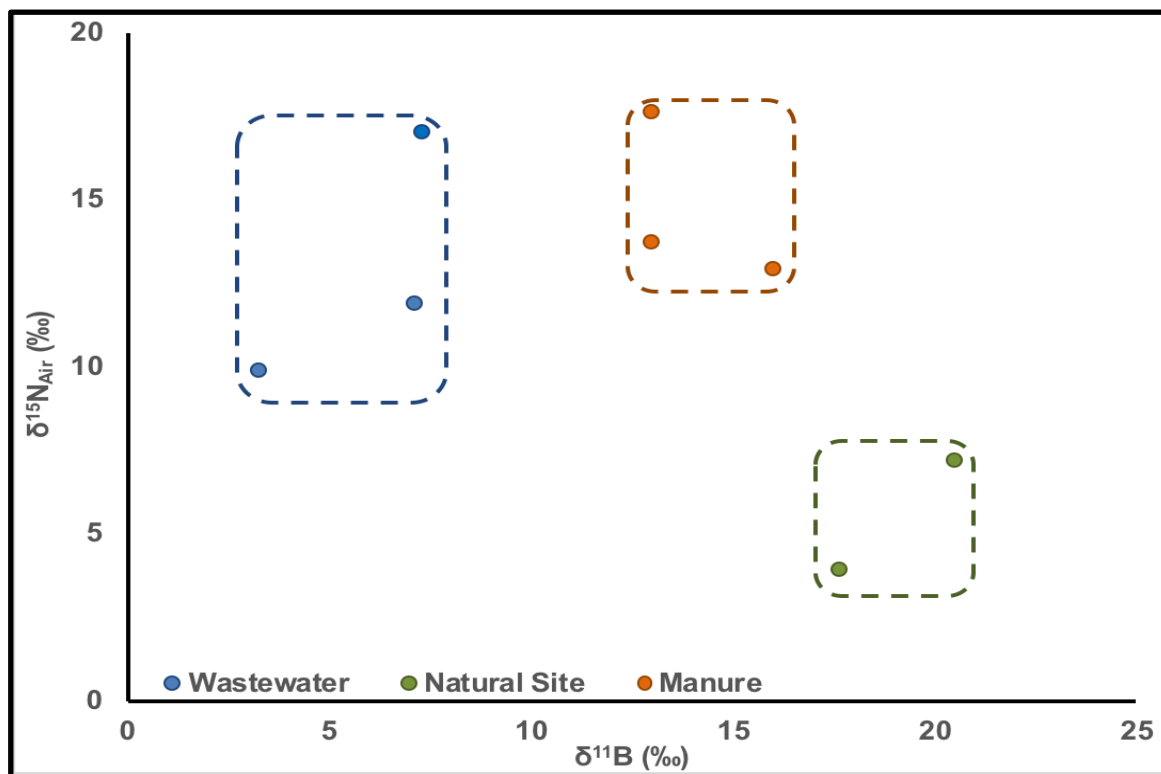


Figure 17a. Boron ( $\delta^{11}\text{B}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) from wastewater, manure, and natural sites.

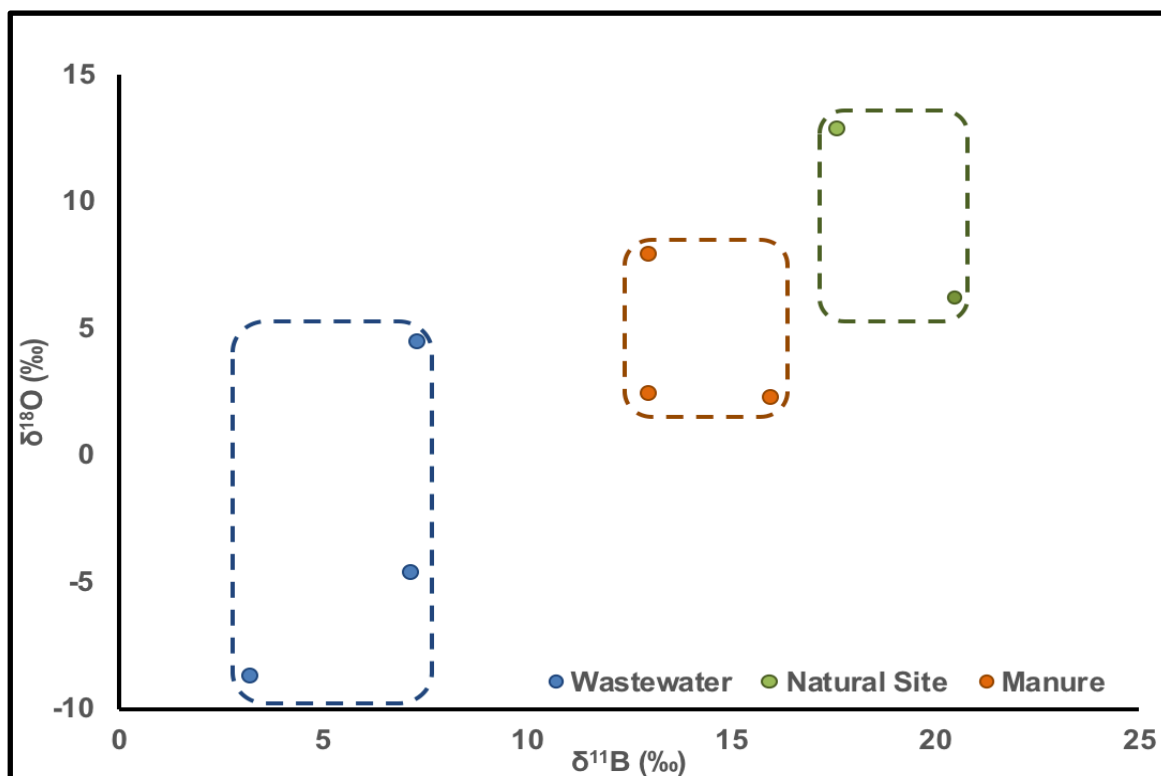


Figure 17b. Boron ( $\delta^{11}\text{B}$ ) and oxygen ( $\delta^{18}\text{O}$ ) from wastewater, manure, and natural sites.

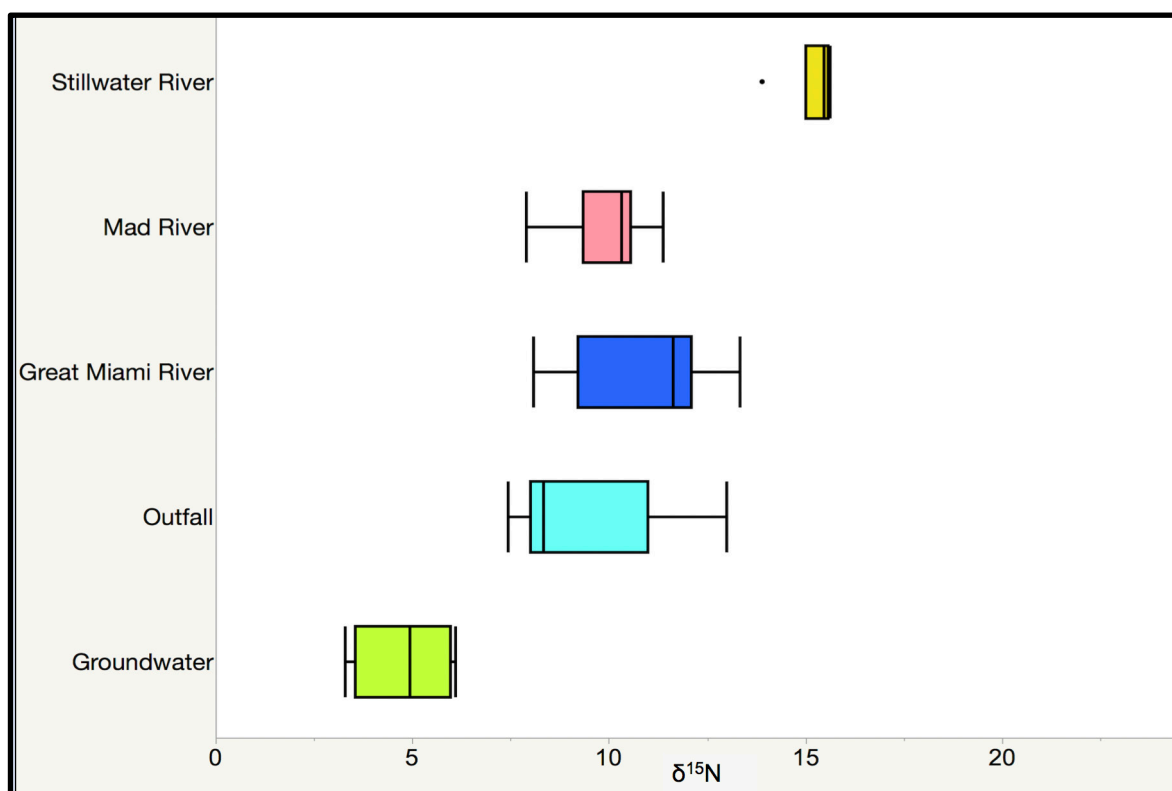
Our boron analysis showed unique  $\delta^{11}\text{B}$  ranges for different sources. For WWTP, isotopic ranges were between +3.25‰ and +7.16‰ for  $\delta^{11}\text{B}$  and between -8.7‰ and -4.6 ‰  $\delta^{18}\text{O}$ . Natural sites isotopically ranged between +10.89‰ and +17.64‰ for  $\delta^{11}\text{B}$  and between -1.2‰ and +14.7 ‰  $\delta^{18}\text{O}$ . Outfalls had a broad range of +6.8‰ and +17‰ for  $\delta^{15}\text{N}$  and between +0.1‰ and +12.9‰ for  $\delta^{18}\text{O}$ . Lastly, manure samples had isotopic ranges between +13.0‰ and +16.0‰ for  $\delta^{11}\text{B}$  and between +2.3‰ and +7.9‰ for  $\delta^{18}\text{O}$  (Fig. 16a and 16b).

As shown in Figure 15a,  $\delta^{15}\text{N}$  of human and animal waste overlaps, so further analysis of boron isotopes ( $\delta^{11}\text{B}$ ) is used to distinguish anthropogenic sources from natural sources. When plotted against  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ , the  $\delta^{11}\text{B}$  creates clear demarcations between contaminant sources, eliminating the overlap left behind by strictly  $^{15}\text{N}\text{-NO}_3^-$ ,  $^{18}\text{O}\text{-NO}_3^-$  analysis (Fig. 17a and 17b).

### 3.2. GMRW Groundwater and Surface Water Isotopic Signatures

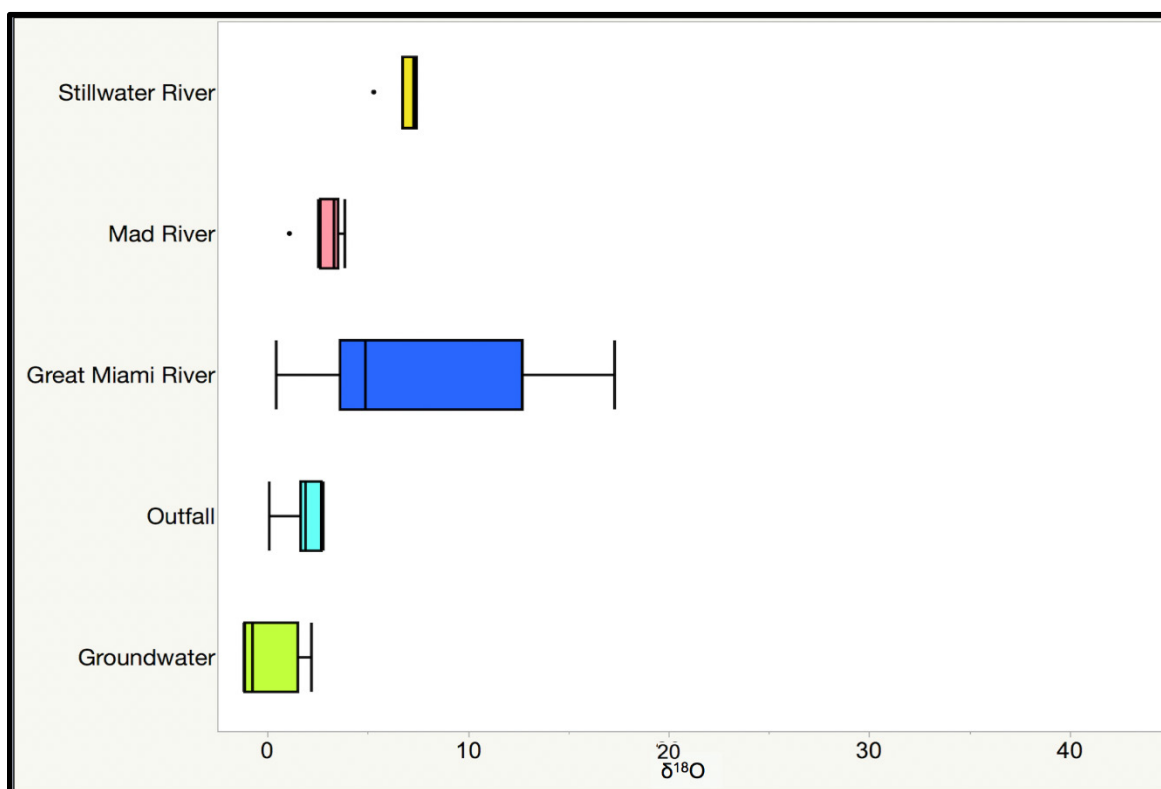
Additional sampling conducted on groundwater, outfalls, and surface water in Fall 2018 successfully shows again that different nitrate sources will exhibit different isotopic compositions. Isotopic signatures for the Great Miami River, Mad River, Stillwater River and the categories of groundwater and outfalls were created for comparison. Values of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  were plotted based on source. Additionally,  $\delta^{15}\text{N}$  values were plotted against  $\delta^{18}\text{O}$  to understand where the data fit into previously published isotopic signature fields for the different nitrate contaminants. The dual tracing method was successful in providing a wider range of separation of isotopic signatures since  $\delta^{15}\text{N}$  values from different sources can overlap. Overall, the data resulting from the Fall 2018 sampling, in conjunction with summer 2017 isotopic signatures, provided insight into the dominant source of nitrate contamination in surface water and groundwater sources.

For the Stillwater River, isotopic ranges were tightly between +15.39‰ and +15.63‰ for  $\delta^{15}\text{N}$  and between 7+.21.7‰ and +7.43‰  $\delta^{18}\text{O}$ . Mad River samples isotopically ranged between +9.28‰ and +11.39‰ for  $\delta^{15}\text{N}$  and between +2.63‰ and +3.87‰  $\delta^{18}\text{O}$ . Great Miami River samples had a broad range of +9.90‰ and +13.34‰ for  $\delta^{15}\text{N}$  and between +3.53‰ and +6.91‰  $\delta^{18}\text{O}$ . Outfall samples had more enriched isotopic ranges +7.43‰ and +11.94‰  $\delta^{15}\text{N}$  and between +0.44‰ and +12.72‰  $\delta^{18}\text{O}$ . Lastly, groundwater isotopically ranges between +4.2‰ and +11.9‰ for  $\delta^{15}\text{N}$  and between +9.8‰ and +15.4‰  $\delta^{18}\text{O}$  (Fig. 18a and 18b). While there is overlap among the isotopic signatures for both nitrogen and oxygen, these ranges ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) provide insights into the source of nitrate contamination when compared to our previous regional isotopic signatures established during 2017 sampling and previously published work.



**Figure 18a.** Nitrogen isotopic signatures for river samples based on source, outfalls, and groundwater.





**Figure 18b.** Oxygen isotopic signatures for river samples based on source, outfalls, and groundwater.

#### 4. Discussion

Data successfully displays distinct isotopic signatures for natural and anthropogenic contamination sources. Previously published values corroborate the success of this case study's dual isotope approach. Our results fall within a reasonable range of previous work and shows some regional variability (Table 2). Our results show a distinct low  $\delta^{15}\text{N}$  for commercial synthetic fertilizers ( $0.4 \pm 4\text{‰}$ ) and high  $\delta^{15}\text{N}$  for animal and human waste ( $13.0 \pm 1.3\text{‰}$ ) (Table 2 and 3). Additionally, our runoff samples indicate a higher  $\delta^{15}\text{N}$  for precipitation ( $>+5\text{‰}$ ) as opposed to  $-3\text{‰}$  (Table 2 and 3).

Sample Source	<u>Our Data</u>	
	$\delta^{15}\text{N}$ (‰)	$\delta^{18}\text{O}$ (‰)
Commercial Fertilizer	+3 to +13	> 0
Animal or Human Waste	+9 to +14	-4 to +8
Precipitation	> +5	>+30
Organic Nitrogen/ Soil Nitrogen	+4 to +12	-1 to +16
Great Miami River	+8 to +13	+4 to +13
Mad River	+8 to +11	+1 to +4
Stillwater River	+15.4 to +15.6	+7.2 to +7.43
Groundwater	+3 to +6	-1 to +2
Outfalls	+0.5 to +10	+8 to +12

**Table 3.** Summary table of isotopic ranges ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) collected from different land usages in 2017, and both surface and groundwater in 2018.

#### 4.1. Dual Isotope Approach

Single isotope analysis is limited in its ability to provide distinction between contaminant sources due to their overlapping isotopic values. This would fail to provide a chemical basis for contaminant tracing. The advantage of dual isotope analysis is that oxygen ( $\delta^{18}\text{O}$ ) is a more conservative isotope than  $\delta^{15}\text{N}$ , so it aids in creating a wider range of separation for isotopic signatures (Kendall, 1998). Analysis of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  ( $^{15}\text{N}\text{-NO}_3^-$ ,  $^{18}\text{O}\text{-NO}_3^-$ ) was first used for the Sagamon River in 1972 by Kohl et al. In the 1990s,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  analysis grew in popularity as it became recognized as a useful way to identify sources, relative contributions, and pathways of nitrate contaminants in water (Ohte, 2013). This technique used in our case study proves successful since different sources have exhibited different isotopic compositions or unique “fingerprints” (Table 2, Figure 15a&b). The distinction of these isotopic end members serves as a basis for identifying the different contaminant sources.

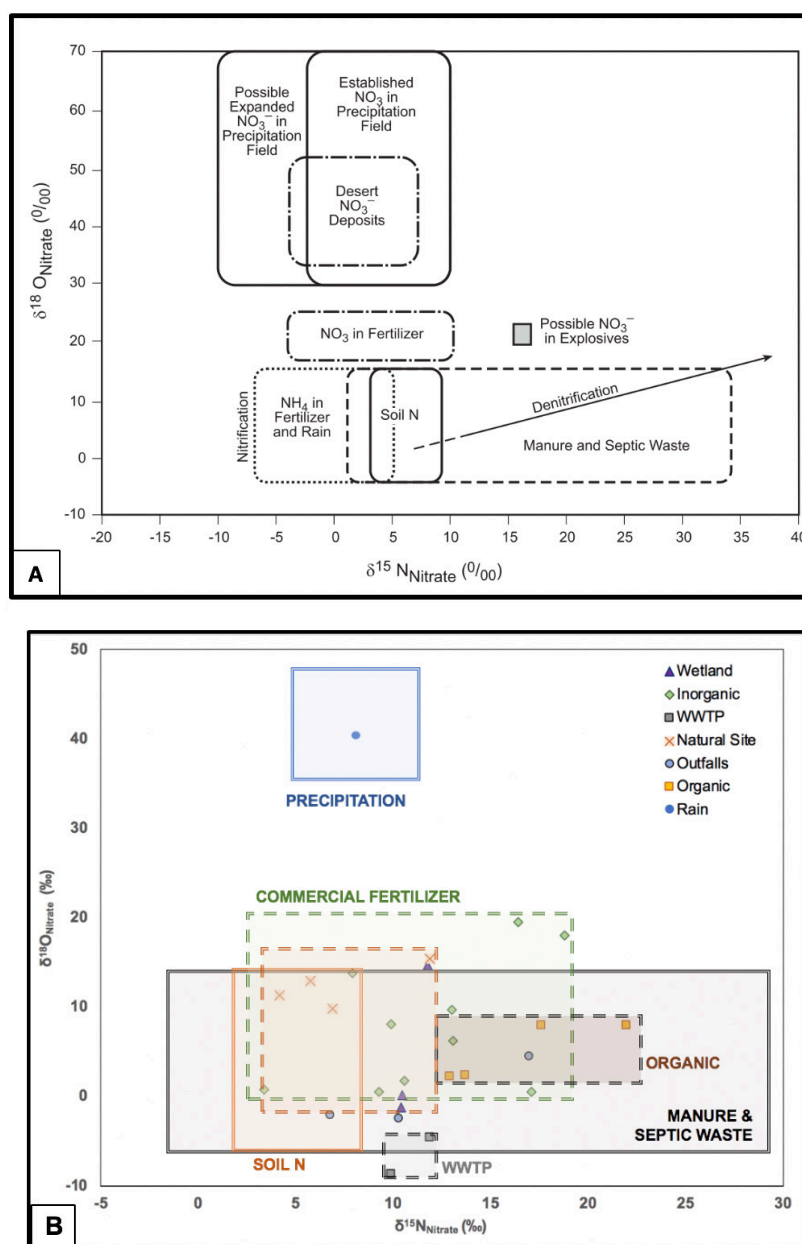
The analysis of  $\delta^{18}\text{O}$  in addition to  $\delta^{15}\text{N}$  is important because it facilitates the separation between animal and human waste, as well as the separation among all other sources.. The overlap of animal and human waste can serve as complications in data interpretation. An enrichment of  $\delta^{15}\text{N}$  can occur due to denitrification processes, resulting in  $\delta^{15}\text{N}$  values overlapping with other nitrate sources (Fukada et al., 2003). Analysis of  $\delta^{15}\text{N}$  alone will not make a strong case for differentiation among different nitrate contaminant sources (Eppich et al., 2012). While some nitrate sources may have a distinct  $\delta^{15}\text{N}$  range, others overlap and cannot be distinguished without additional methods. These sources that often overlap in  $\delta^{15}\text{N}$  values have unique  $\delta^{18}\text{O}$  ranges.

#### *4.2. Comparison against previous data*

In Figure 20b, the transparent boxed fields indicate previously published ranges and isotopic fingerprints for precipitation, manure and septic waste, and soil nitrogen. The dashed boxes represent isotopic fields derived from our collection of local samples (Fig. 15a&b). These dashed boxes were created with reference to Motzer (2006) data, but zones were adjusted based on our study's isotopic compositions established through our "fingerprinting" data collected summer 2017. Our data falls within a reasonable range of previously collected data for nitrogen and oxygen nitrate isotope signatures for various contamination sources, and they show regional variability.

Regional variability for our isotopic signatures could be explained with climate factors such as precipitation and both air and water temperatures. Spatial factors such as the presence and amount of impervious surfaces, agricultural land, and forest areas. These characteristics can affect bacterial activity, nitrogen transformation processes, and isotopic fractionation, and this can ultimately modify isotopic compositions of samples (Xu et al., 2016).

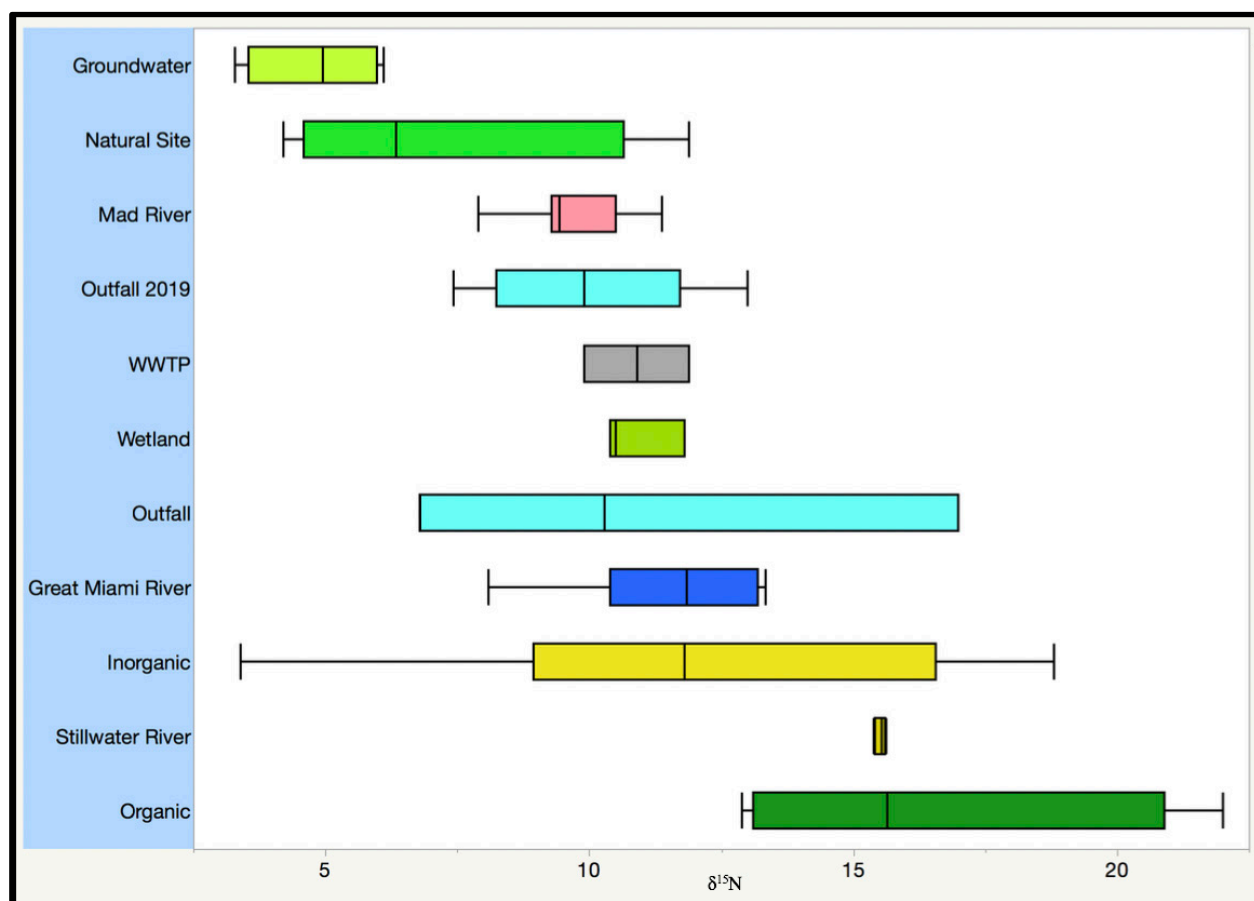
The forested and prairie-like regions sampled along with the results of our wetland data show that local soil nitrogen or organic nitrogen indicates the greatest shift. It has some overlap with previously selected work but our  $\delta^{15}\text{N}$  ranges between +4 to +12  $\delta^{18}\text{O}$  ranges between -1‰ to +16‰, whereas previous  $\delta^{15}\text{N}$  ranges between +4 to +9  $\delta^{18}\text{O}$  ranges between -4‰ to +1‰ (Table 2, Figure 19B).



**Figure 19. A.** Previously published summary table of isotopic ranges ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) for different nitrate sources (Source: Motzer, 2006) **B.** Summary table of isotopic ranges ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) for different nitrate sources based on sample collection during Summer 2017. The solid border boxes indicate pre-established isotopic fields from previous publications, while the dashed line boxes indicate this study's findings (Source: Motzer, 2006).

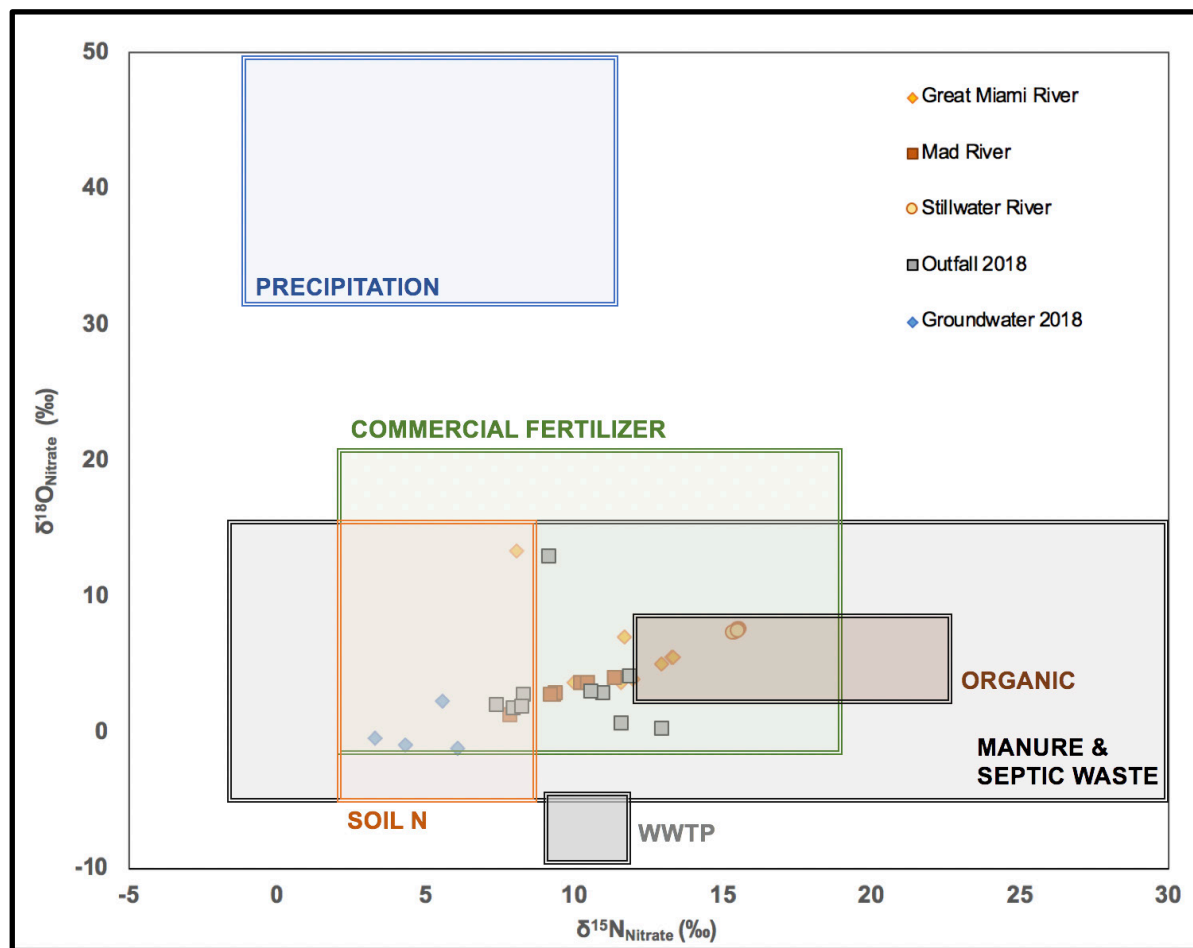
### 4.3 Data Compilation of Summer 2017 and Fall 2018

When compared to the regional isotopic signatures for nitrate contaminants established from summer 2017 samples, surface and groundwater  $\delta^{15}\text{N}$  distinctly overlap. From this we can infer what nitrate source is driving river, outfall, and groundwater contamination. River samples  $\delta^{15}\text{N}$  collected from the Great Miami River, Mad River, and Stillwater River values lies within a range of human and animal waste, +9‰ to +22‰. Outfall and groundwater  $\delta^{15}\text{N}$  values suggest that the nitrates might have been derived from soil organic matter or synthetic fertilizers, +4‰ to +17‰. These inferences suggest a strong correlation between anthropogenic action and nitrate contamination.



**Figure 20.** Nitrogen isotopic ranges ( $\delta^{15}\text{N}$ ) for different nitrate sources from Summer 2017 sample collection and Fall 2018 collection of surface and groundwater samples.

#### 4.4. Surface and Groundwater Isotopic Composition



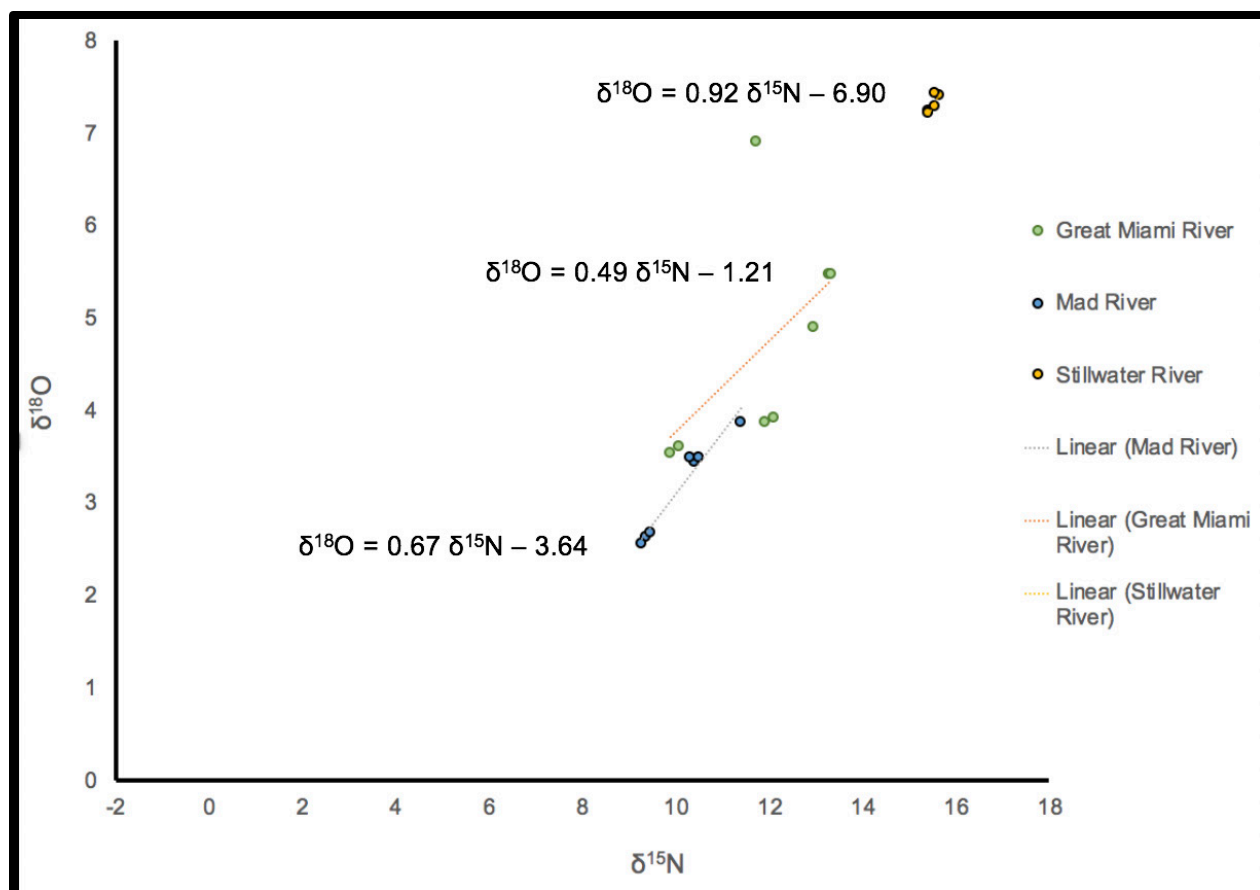
**Figure 21.** Summary of isotopic ranges ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) for different nitrate sources based on sample collection during Summer 2017. The solid border boxes indicate pre-established isotopic fields from summer 2017 “fingerprinting” study (Source: Motzer, 2006).

When  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of river, groundwater, and outfall samples are plotted, it verifies our inferences about nitrate contaminant sources based on  $\delta^{15}\text{N}$  alone. Groundwater and some outfall sites fall within the overlapping range of organic soil nitrogen and synthetic fertilizer. Most outfall sites are dispersed within the range of synthetic fertilizer. Meanwhile, the river samples can be found within the ranges of both human (manure and septic waste) and organic animal waste.

Since nitrate-rich wastewater treatment effluent is released into the rivers post-treatment, it would make sense that nitrate found in these samples are within the the human waste isotopic signature. Outfall sites are areas of water discharge from areas including runoff, pipes, and municipal subsurface drainage. Both runoff and groundwater interact with the soil, absorbing any nutrients such as those in excess amounts from synthetic fertilizer that may be present on residential lawns and gardens or municipal areas. Since nitrate is highly soluble and mobile in nature, enriched runoff is a notable contributor to nitrate contamination. This runoff is carried through municipal infrastructure and can be collected at outfall sites.

#### *4.5. Denitrification*

Plotting  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of nitrate can be done to determine whether denitrification or the biological transformation of nitrate to molecular nitrogen has occurred. When observing both isotopes, the ratio of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  changes as denitrification occurs because the remaining nitrate becomes isotopically heavier and the ratio is about 2:1 (Kendall, 1998). When our surface water samples are plotted, there is a linear trend visible, but it doesn't have an exact 2:1 ratio (Fig.22). Denitrification cannot be definitively seen. The nitrate concentration, temperature, and discharge could be acting as factors in nitrogen transformation in river samples (Laursen and Seitzinger, 2002). These could be relevant factors that help to explain why our samples are not clearly showing a 2:1 ratio. Parameters such as sample filtration and refrigeration were used to minimize denitrification and sample compromise, but some natural denitrification before sampling likely occurred due to the presence of anaerobic bacteria in the water samples that were still active until they were immobilized via refrigeration.



**Figure 22.** Linear trends in isotopic composition ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) of surface water samples.

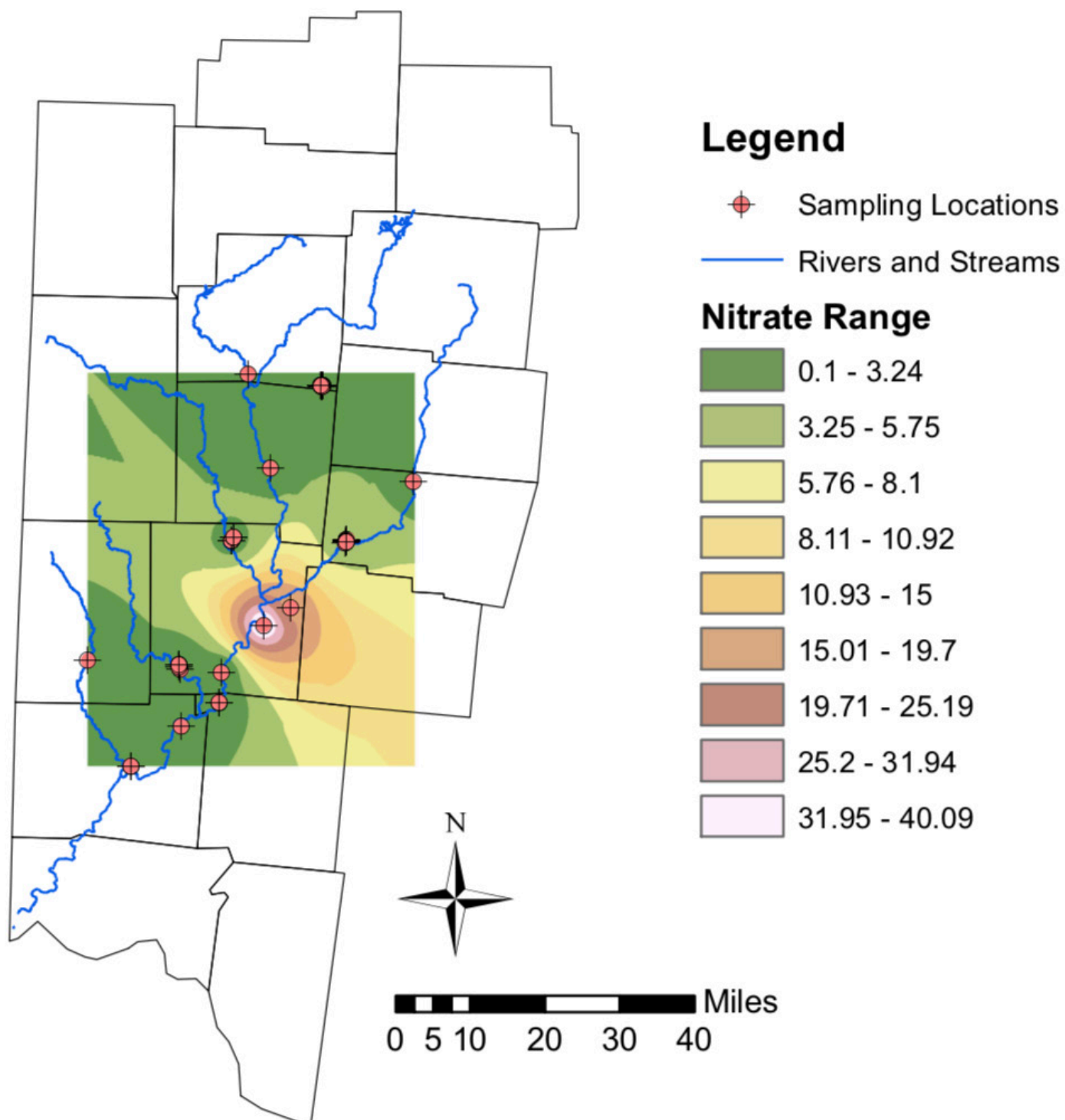
#### 4.6. GIS Analysis

Nitrate concentration data collected throughout this study from water samples along the Great Miami River, Mad River, and Stillwater River as well as groundwater and outfall sites. Samples were taken during the fall season and are not reflective of a long-term average, so it is only a snapshot. GIS analysis was used to create a nitrate concentration contours. This analysis reveals that there is a nitrate contamination area spanning the area surrounding the confluence of the Great Miami River, Mad River, and Stillwater River towards downstream (Fig. 23). As nitrate contamination is brought south from the headwaters of these rivers, they accumulate and concentrate primarily in the Greater Dayton Metropolitan area, which holds a population of approximately 140,371 people (Statistical Atlas, 2019).

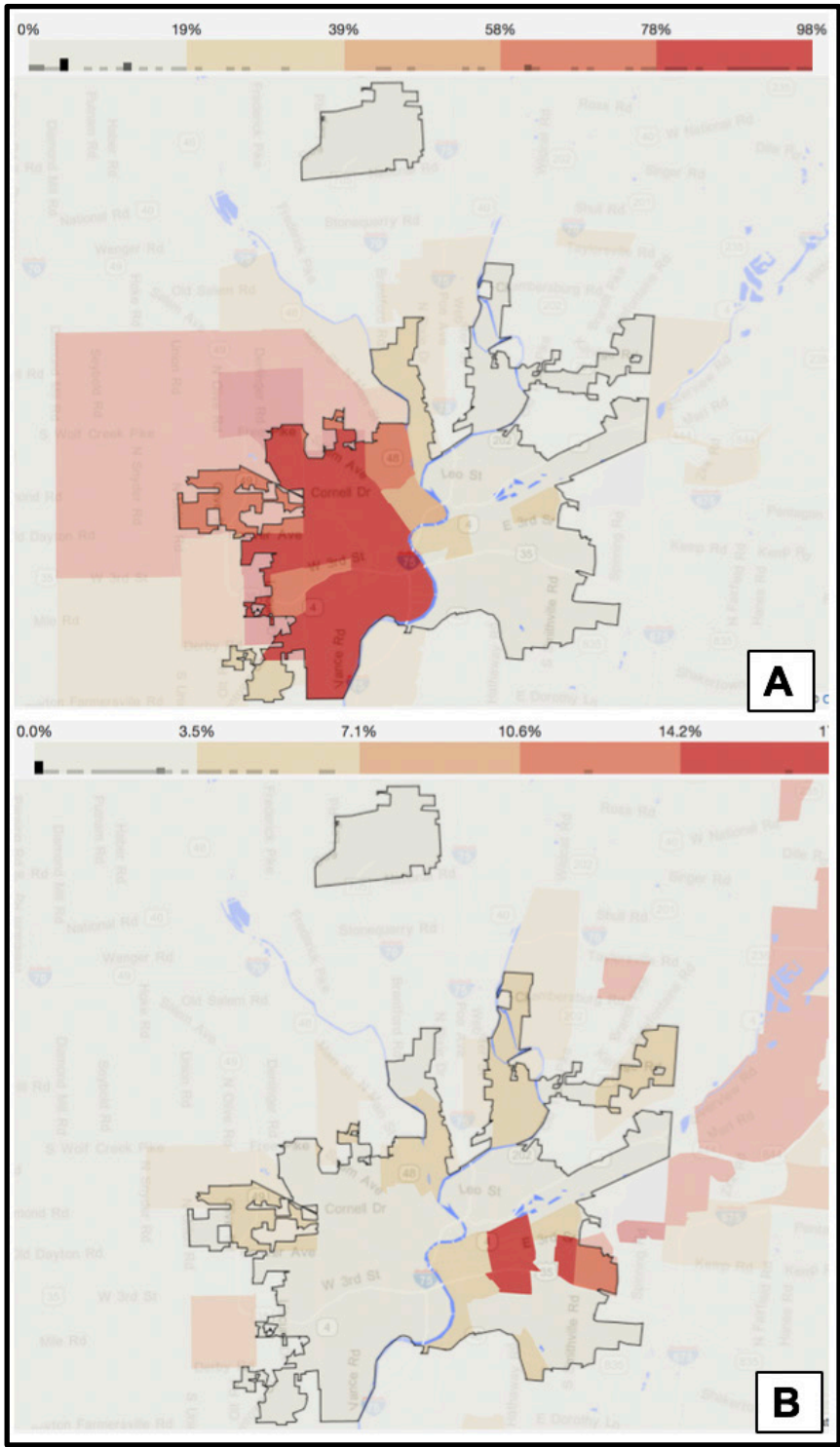


Nitrate concentrations in this contamination area ranged from 8 mg/L to 40 mg/L at sampling sites. The EPA safe drinking standard for nitrate is 10 mg/L, so these elevated nitrate concentrations are notable. It is a threat to aquatic ecosystems and the organisms within them due to the threat of eutrophication from nutrient enrichment. The highest nitrate contamination points were from outfall samples and isotopically they fall within the range of synthetic fertilizers.

In terms of the demographics of the populations that are located within this high nitrate contaminant area, the west side of the Great Miami River located within this zone is 60 to 99% black population (Fig. 24a) (Statistical Atlas, 2019). The east side of the Great Miami River which is located within this contaminant zone has a more heterogeneous population, but there are areas containing up to 18% Hispanic population (Fig. 24b)(Statistical Atlas, 2019). Nitrate concentrations are high in the surrounding area of the 3-river's confluence, but since this type of contamination is a result of natural phenomena it may not be a case of injustice despite correlation to a larger minority population.



**Figure 23.** Nitrate concentration levels within the GMRW.



**Figure 24. A.** Demographics map of the Black population within the Dayton metropolitan area. **B.** Demographics map of the Hispanic population within the Dayton metropolitan area (Map Source: Statistical Atlas, 2019)

#### *4.7. Nutrient Management Strategies*

Based on this study, anthropogenic input to nitrate contamination is mainly comprised of the following: human waste (municipal and septic), animal waste, and synthetic fertilizer. In order to mitigate excessive nutrient contributions such as nitrate, changes need to be made. Synthetic fertilizers such as those used on residential lawns and gardens, as well as commercial agricultural fertilizers are typically applied in excess. The excessive nutrients such as nitrate are highly soluble and easily mobilized upon application of water such as precipitation. Runoff enriched with nutrients from residential and agricultural sites may continue to travel to surface water due to increased impervious surfaces, or it may eventually be reabsorbed into local groundwater resources. While fertilizer and manure can boost productivity of crops or plants, it needs to be applied more wisely. Being conscious of the amount of synthetic fertilizer or manure used at a site as well as limiting usage of highly-enriched commercial fertilizers, could help reduce the amount of nitrate carried away by runoff, therefore lowering the amount of nitrate that seeps into groundwater sources.

The GMRW could benefit from local legislation comparable to those enacted in the Lake Erie Western Basin Watershed in July 2015. Fertilizer and manure application should be restricted during the following: (1) snow-covered or frozen soil, (2), when the top soil layer, up to 3 inches, is saturated from precipitation, (3) when local weather forecast in the application area has greater than a 50% change of precipitation within the next 2 days, exceeding half an inch of precipitation within a 24-hour period (Ohio State University, 2018).

Furthermore, while local WWTP meet effluent standards set by Ohio State and NPDES permits, the effluent being released is still affecting the general health of the region's surface water resources. The nutrient-related impairment of the Great Miami River, Mad River, and

Stillwater River needs to be addressed. Lowering the local acceptable level of nitrate in wastewater effluent at major municipal WWTP should be the first step in eliminating nutrient-related impairment of our rivers. While the effluent itself may be deemed safe for the marine life and habitat upon reentry into surface water sources, as nutrients bioaccumulate in the water and downstream, this effluent poses a greater threat to these ecosystems. Effluent limits established by the Clean Water Act and local legislation need to be reevaluated, and regulations should be made more strict as the current standards for effluent are still allowing damage to regional surface water sources. These local issues of nutrient enrichment are then amplified downstream, so attending to the sources upstream in our area is an important step for the community to take.

#### 4.8. Conclusion

The GMRW is affected by nitrate contamination and local monitoring stations have shown elevated nitrate concentrations in both surface and groundwater. It is important to address the issue and its sources, especially the anthropogenic contributions such as synthetic fertilizers, wastewater effluent, and animal manure, since nitrate contamination can have negative effects on human health, aquatic organisms, and trophic balance.

The isotopic composition of nitrate contaminant sources within the watershed have been established through this study using a stable isotope dual tracing method of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  Analysis ( $^{15}\text{N}\text{-NO}_3^-$  and  $^{18}\text{O}\text{-NO}_3^-$ ) to trace nitrate contamination to its regional sources. We found that each has a unique “fingerprint” and range that can be used to chemically identify it in future studies. Our work’s accuracy can be corroborated by previous case studies and research. Additionally, a distinction between anthropogenic and natural sources of nitrate contamination can be found using  $\delta^{11}\text{B}$  analysis.

Surface water analysis using the  $\delta^{15}\text{N}$  of samples from the Great Miami River, Mad River, and Stillwater River revealed that local rivers are being contaminated by nitrate sourced at animal manure or human waste. Meanwhile, the nitrates found in groundwater are thought to have been derived from soil organic matter or synthetic fertilizer. Mapping analysis reveals a contamination zone within the GMRW; it spans the confluence of the three rivers and extends downstream. This reveals a bioaccumulation of nutrients affecting our local waterways, likely due to nutrient inputs at our headwaters. These initial findings lay the groundwork for more comprehensive future studies about nitrate contamination in the GMRW.

With a global population projected to increase exponentially, it is vital that food production needs are met. While synthetic fertilizers may be necessary to boost crop productivity to achieve this goal, it is important to apply it in a more strategic manner. In the same way, growing populations also means increased waste production. Wastewater treatment plants are necessary for the maintenance of human health, but the nutrient contents of effluent and its dumping sites need to be reviewed.

Nutrient management strategies for fertilizer and manure application could be better calibrated to the GMRW using the results from this study. The Greater Dayton area operates at the intersection of an urban and residential area surrounded by agriculture. Notable nitrate sources, such as synthetic fertilizers for agricultural and residential use, animal manure, and wastewater effluent, are key components to the functionality of the area and its population. This is why increased awareness of nitrate contamination within the area could better serve the community towards a goal of more sustainable practices.

#### *4.9. Future Work*

This study operated under limited funding which resulted in a small sample size. Future work could greatly benefit from further sampling along different land usage sites as done for the Summer 2017 “fingerprinting”. This would allow for a more accurate average and depiction of regional isotopic signatures or fingerprints for local contaminant sources. Further surface and groundwater sampling done year-round during both low and high-river flow would also be beneficial. These steps would allow for the production of a higher resolution data set that could create a more detailed case study of nitrate contaminants in the GMRW. Additional boron sampling, specifically for surface water samples should be a priority as current analysis suggests

that the isotopic signature from the Great Miami, Mad, and Stillwater River coincides with the isotopic signatures of human or animal waste.

Further boron analysis would be able to distinguish whether the nitrate contaminants found in local surface water is a primary result of animal or anthropogenic source. The regional isotopic signatures for each contaminant source within the watershed could also be utilized to quantify the contribution of each nitrate source. The results of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  analysis can be used to quantify nitrate source contributions to water based on the idea that changes in isotopic composition are result of mixing between two or more sources with known isotopic composition. This is a basic isotope mass balance mixing model. This model has successfully been utilized to quantify approximate nitrate contributions from various sources such as farm land, atmospheric deposition, and uncultivated soil in different catchments (Xu et al., 2015).

Additionally, further spatial analysis of the Dayton area utilizing nitrate concentrations and census data should be conducted. This research would help evaluate if minority populations are disproportionately affected by nitrate contamination in the Dayton area. This research provides baseline information on the nitrate contamination in the watershed and provides the foundation for more thorough analysis.



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## Appendix I: Nitrogen, Oxygen, Carbon, and Boron Isotope data

## A. Summer 2017 Water Sample Data

Sample Name	Form	$\delta^{15}\text{N}_{\text{Air}}$ (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ (‰)	$\delta^{13}\text{C}$	B (ppb)	$\delta^{11}\text{B}$
170525-W1	Water	9.3	0.5			
170525-W3	Water	9.9	8.1			
170525-W4	Water	10.4	-1.2			
170525-W6	Water	10.5	0.1		56	10.89
170525-W7	Water	20.8	8.8			
170530-W8	Water	17.1	0.5			
170530-W9	Water	10.6	1.7			
170530-W10	Water	13.1	6.2			
170601-W11	Water	11.9	-4.6		152	7.16
170601-W12	Water	9.9	-8.7		223	3.25
170907-W14	Water	17	4.5		165	7.33
170918-W16	Water	8.1	40.4			
170524-1-2W	Water	13.0	9.7			
170524-3-4W	Water	7.9	13.8			
170524-5-8W	Water	3.4	0.7			
170525-11-12W	Water	11.9	15.4			
170524-15-16W	Water	11.8	14.7			
170525-19-20W	Water	6.9	9.8			
170525-21-22W	Water	4.2	11.3			
170530-23-24W	Water	22	7.9			
170530-27-28W	Water	18.8	18			
170530-29-30W	Water	16.4	19.5			
170530-31-32W	Water	5.8	12.9			
C33W	Water	13.7	2.4			
P34W	Water	17.6	7.9			
C35W	Water	12.9	2.3			
171201-W17	Water	10.4	-2.6			
171201-W19	Water	6.8	-2.1			

## B. Summer 2017 Soil Sample Data

Sample Name	Form	$\delta^{15}\text{N}_{\text{Air}}(\text{‰})$	$\delta^{18}\text{O}_{\text{VSMOW}}(\text{‰})$	$\delta^{13}\text{C}$	B (ppb)	$\delta^{11}\text{B}$
170524-1	Soil	4.3		-21.8		
170524-2	Soil	7.2		-22.2		
170524-3	Soil	2.9		-25.3		
170524-4	Soil	5.2		-24.9		
170524-5	Soil	-0.4		-25.3		
170524-6	Soil	3.7		-25.2		
170524-7	Soil	4.1		-23.4		
170524-8	Soil	6.3		-20.7	5.3	15.71
170524-9	Soil	7.2		-20.8		
170524-10	Soil	5.8		-20	0.95	20.51
170524-11	Soil	5.9		-21.2		
170524-12	Soil	5.6		-19.4		
170524-13	Soil	6.1		-18.4		
170524-14	Soil	1.5		-25.1		
170525-15	Soil	4.5		-25.9		
170525-16	Soil	5.8		-25.2		
170525-17	Soil	-0.3		-26.6		
170525-18	Soil	2.4		-26.2		
170525-19	Soil	2.5		-24.7		
170525-20	Soil	2.9		-24.6		
170525-21	Soil	1.6		-26		
170525-22	Soil	4.1		-25.9		
170530-23	Soil	5.7		-16.9		
170530-24	Soil	5.7		-24.4	3.57	10.12
170530-25	Soil	4		-25.4		
170530-26	Soil	7.3		-23.1		
170530-27	Soil	4.7		-20.8		
170530-28	Soil	5.7		-22.8		
170530-29	Soil	6.4		-21.3		
170530-30	Soil	6.4		-22.2		
170530-31	Soil	3.9		-25.7	1.08	17.64
170530-32	Soil	5.8		-25.4		
C33_soil	Soil	8		-20.8	16.7	13
P34_soil	Soil	10.8		-20.7	45.2	13
C35_soil	Soil	1		-15.1	13.4	16

## C. Fall 2018 Data

Sample Name	Type	$\delta^{15}\text{N}_{\text{Air}}$ (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ (‰)
W1	Ground water	5.6	2.2
W2	Ground water	6.1	-1.2
W3	Ground water	4.3	-1
W4	Ground water	3.3	-0.5
W5	River	13.9	5.3
W6	River	11.6	3.6
W7	River	7.9	1.1
W8	River	11	2.7
W9	River	10.6	2.8
W10	River	13	0.1
091618-W17	Outfall	11.65	0.44
092218-W19	Outfall	11.94	4.01
092318-W26	Outfall	-0.03	17.30
092318-W28	Outfall	9.21	12.72
091618-W11	River	13.27	5.47
091618-W12	River	11.72	6.91
091618-W13	River	13.34	5.48
091618-W18	River	12.97	4.90
092218-W20	River	11.89	3.85
092218-W20 REP	River	12.09	3.92
092318-W29	River	8.09	13.21
092318-W30	River	9.90	3.53
092318-W30 REP	River	10.09	3.60
092318-W27	River	-0.59	16.76
092218-W21	Outfall	10.61	3.66
091518-W10	Outfall	10.55	3.40
091518-W10 REP	Outfall	10.38	3.26
092218-W22	River	10.42	3.44
091618-W14	River	9.37	2.63
091618-W15	River	9.44	2.66
091618-W16	River	9.28	2.56
092218-W23	River	10.29	3.49
092218-W24	River	10.50	3.49
092218-W25	River	11.39	3.87
091518-W5	River	15.42	7.23
091518-W6	River	15.63	7.41
091518-W7	River	15.57	7.43
091518-W8	River	15.39	7.21
091518-W9	River	15.54	7.29
091418-W1	Outfall	7.43	1.91
091418-W2	Outfall	8.01	1.64
091418-W3	Outfall	8.35	2.61
091418-W4	Outfall	8.32	1.79

**Appendix II: Nitrate concentration data**

<b>Sample</b>	<b>Type</b>	<b>NO<sub>3</sub>- (mg/L)</b>
091418-W1	Outfall	3.22
091418-W2	Outfall	8.55
091418-W3	Outfall	5.32
091418-W4	Outfall	2.16
091518-W5	River	2.64
091518-W6	River	2.65
091518-W7	River	2.44
091518-W8	River	2.7
091518-W9	River	2.67
091518-W10	River	1.08
091618-W11	River	2.74
091618-W12	River	1.58
091618-W13	River	2.78
091618-W14	River	2.26
091618-W15	River	2.35
091618-W16	River	2.36
091618-W17	Outfall	10.2
091618-W18	River	2.56
092218-W19	Outfall	2.01
092218-W20	River	2.02
092218-W21	River	2.35
092218-W22	River	2.36
092218-W23	River	2.01
092218-W24	River	0.169
092218-W25	River	0.194
092318-W27	River	0.39
092318-W28	Outfall	0.361
092318-W29	River	1.83
171201-W17	Outfall	40.1
171201-W19	Outfall	18.8

### Appendix III: Physio-chemical Data from Field Work

Sample Name	Depth (ft.)	Turbidity (NTU)	DO (mg/L)	pH	ORP (mv)	Temp (°C)	SC (µS/cm)	Salinity (psu)	TDS (ppt)
170525-W1	0.18	73.76	6.38	7.73	162.73	20.11	214.38	0.102	0.159
170525-W2	0.22	62.45	1.32	6.92	-15.9	15.72	564	0.28	0.37
170525-W4	1.29	85.25	4.24	7.4	115.8	12.32	637.73	0.31	0.414
170525-W5	0.92	1.139	4.2	7.35	140.08	14.79	594.7	0.29	0.38
170525-W6	1.82	18.13	4.95	7.57	-28.51	15.79	584.46	0.28	0.38
170525-W7	0.42	18.87	8.53	8.18	131.45	17.07	492.77	0.23	0.32
170530-W8	1.32	7.66	10.83	8.03	104.79	17.08	618		0.4
170530-W10	0.25	1.14	8.83	7.37	146.77	14.28	558.58	0.27	0.36
170601-W11	1.33	1.67	8.04	7.68	124.45	18.58	1246.93	0.63	0.81
170601-W12	2.28	2.21	4.01	7.44	135.99	19.2	1405.79	0.71	0.91
W1	30.6		9.55	7.24		12.8	538		
W2	30.6		9.4	7.23		12.7	538		
W3	8.07		3.44	7.13		15.3	664		
W4	8.07		3.63	7.13		15.8	664		
091418-W1			6.9	8.33	203.2	21.5	744.2		
091418-W2			7.4	8.27	200.3	21.7	744.1		
091418-W3			5.8	8.29	165.5	21.8	744.4		
091418-W4			6.3	8.32	167.8	22.1	744.5		
091518-W5			6.6	8.27	163.1	22	744.4		
091518-W6			6.2	8.15	162	21.4	745.1		
091518-W7			6.7	8.09	165	21.7	743.9		
091518-W8			6.4	8.11	165.2	22	744.1		
091518-W9			6.2	8.16	165.6	22.3	744.5		
091518-W10			6.6	8.22	161	22.8	744.5		



### Appendix IV: Loss on Ignition data

Sample	Wt. of crucible	wt. crucible + sample (g)	sample wt. (g)	wt. crucible + sample dry (g)	Water content (%)	wt. crucible + sample (g)	Organic Matter Content (%)	wt. crucible + sample heated (g)	Carbonate content (%)
1	6.25	7.22	0.97	7.10	1.65	7.07	0.47	7.06	0.11
2	5.70	6.66	0.97	6.51	2.28	6.47	0.53	6.47	0.13
3	6.00	6.88	0.89	6.71	2.48	6.65	0.88	6.64	0.16
4	6.69	7.69	0.99	7.50	2.37	7.44	0.84	7.43	0.18
5	6.22	7.16	0.93	7.04	1.61	7.00	0.55	6.98	0.29
6	6.21	7.17	0.96	7.05	1.74	7.01	0.53	6.99	0.34
7	5.91	6.83	0.92	6.70	1.82	6.67	0.51	6.62	0.68
8	6.65	7.63	0.98	7.49	1.89	7.46	0.41	7.32	1.85
9	6.06	7.03	0.97	6.81	3.13	6.74	1.00	6.73	0.16
10	6.43	7.42	0.99	7.24	2.37	7.19	0.70	7.18	0.15
11	6.33	7.29	0.96	7.06	3.14	6.99	1.07	6.98	0.13
12	6.05	7.05	1.00	6.86	2.72	6.79	1.04	6.78	0.15
13	5.64	6.52	0.88	6.28	3.72	6.22	0.85	6.22	0.11
14	6.27	7.23	0.96	7.04	2.66	7.00	0.55	6.99	0.11
15	6.20	7.13	0.92	6.89	3.29	6.82	1.00	6.79	0.52
16	5.88	6.81	0.93	6.60	3.08	6.55	0.83	6.51	0.62
17	6.27	7.21	0.93	6.93	3.88	6.86	0.94	6.85	0.15
18	6.43	7.42	0.99	7.23	2.49	7.18	0.67	7.17	0.14
19	5.61	6.51	0.90	6.23	4.28	6.16	1.13	6.14	0.44
20	6.24	7.18	0.95	6.89	4.14	6.81	1.14	6.78	0.47
21	6.09	7.07	0.98	6.79	4.05	6.73	0.87	6.72	0.07
22	5.35	6.35	0.99	6.11	3.65	6.07	0.67	6.07	0.09
23	6.00	7.00	1.00	6.83	2.33				
24	6.27	7.26	0.98	7.09	2.31				
25	6.24	7.23	0.99	7.08	2.10				
26	5.88	6.86	0.98	6.71	2.23				
27	6.27	7.22	0.95	7.02	2.74				
28	6.43	7.34	0.91	7.15	2.47				
29	5.61	6.60	0.98	6.42	2.62				
30	6.24	7.13	0.89	6.97	2.25				
31	6.09	7.04	0.95	6.87	2.49				
32	5.69	6.54	0.84	6.33	3.23				